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PLANT AND SOIL

International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases

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PLANT AND SOIL

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FOREWORD

At the present time the publishing facilities for research workers in the field of Agricultural Science are very limited; consequently the existing Journals are overcrowded and often one has to wait many months or even more than a year before papers, even of universal importance, can be published.

Moreover, in the smaller countries many scientists in this field are forced to publish the results of their investigations in national Journals of heterogenous contents and in their native language. As a consequence valuable work often remains unknown to scientists abroad. The increasing demand for intensive crop production all over the world, however, necessitates a quick exchange of the results of scientific agricultural research.

Realizing this lack of an appropiate medium of publication, the Editors have designed the new Journal „Plant and Soil" to deal particularly with those branches of research, which are centered around the plant and its relations to the soil. Plant physiology, especially nutrition and biochemistry, and soil science as far as connected with plant nutrition, soil microbiology and soil-borne plant diseases are regarded as forming some kind of a natural unit which the new Journal will attempt to serve. Subjects such as genetics, pure phytopathology, agrohydrology as well as extensive reports on field and fertilizer experiments are outside the scope of the new Journal.

It is the Editors' wish that the new Journal may have its readers amongst agricultural scientists all over the world. Research workers can be assured that their contributions will receive prompt and careful attention and will be considered in order of receipt.

It is the hope of the Editors that the new Journal may contribute to the re-establishment of international intercourse in this important branch of science.

THE EDITORS

Plant and Soil I, no 1 January 1948

DETERMINATION OF BIOTIN IN BEET MOLASSES WITH *NEUROSPORA CRASSA* SHEAR AND DODGE AS A TEST-ORGANISM

by ELIAS MELIN and BIRGITTA NORKRANS

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All microbiological methods for the determination of biotin are based on the fact that the growth-rate of the test-organisms, cultivated on an otherwise optimal basal medium and within a limited range, increases in proportion to the biotin concentration. Since Kögl *et al.* [6], [7], when isolating pure biotin, made their determinations with the aid of yeast-Race M, this method has been modified by several investigators. Snell *et al.* [20] used a small inoculum of yeast and supplied the nutrient solution with vitamin B_6, while Hertz [5], used biotin-free casein hydrolysate, as a result of which both the specificity and the range of biotin determinations made by the yeast-growth-method were increased. This test is most practicable within the range from 1×10^{-4} to 3×10^{-4} γ per culture with a 10 ml medium. Nielsen and Hartelius [13] made corrections for the influence of other growth factors in the samples similar to their earlier procedure [12] with β-alanine. Besides yeast, several biotin-deficient micro-organisms, especially bacteria, have been used in the assay of biotin in body-fluids and tissues. Porter and Pelczer [15] recommended *Staphylococcus aureus*, and since Snell and Williams [21] have found that biotin is the only substance, in addition to the synthetic basal medium, required by *Clostridium butylicum* for luxuriant growth, Peterson *et al.* [14] as well as Lampen *et al.* [9] have used this organism for biotin determinations in natural materials.

West and Woglom [24], [25] have determined the biotin content (amounting to about 0,013 mγ/ml) using a strain of *Rhizobium trifolii*. Nielsen and Hartelius [13] also employed this organism for the determination of small quantities of biotin.

Lactic acid bacteria have been used to a greater extent. Gaines and Stahly[4]) suggested *Leuconostoc mesenteroides* as a test-organism. Shull *et al.*[18]), [19]) introduced the biotin assay with *Lactobacillus casei*. Landy and Dicken[10]), Tomlinson and Peterson[23]) and Williams and Fieger[26]) tried to improve this method but were not able to make it quite specific. Snell and Wright[22]) and Wright and Skeggs[27]) used *L. arabinosus*. This test-organism is more suitable than *L. casei*, because it has less complex growth requirements and can be cultivated in a synthetic medium.

In most cases the growth is measured turbidimetrically, but with the lactic acid bacteria the acid production is an expression of their growth and the quantity of titrable acid is measured.

Robbins and Schmidt[16]) tried to determine the biotin content of, among other things, various sugars with *Nematospora gossypii* Ashby and Nowell. The growth-responses were determined by weighing dried mycelia. The method is tedious, the test-period being, in this case, 15 to 20 days, Kögl's yeast test required 5 hours, Snell's 20 hours, while 72 hours is a usual incubation time for lactic acid bacteria, and 5 days for *Rhizobium trifolii*.

Several species of the ascomycete *Neurospora* are unable to synthesize biotin (Butler *et al.*)[2]). Ryan *et al.*[17]) showed that the growth-rate of two different strains of *Neurospora crassa* is a function of the quantity of biotin-methyl-ester in the range between 0.0128 and 20 mγ per 15 ml. The investigation was made by means of the tube-method, i.e. the progression of mycelial frontiers measured in mm per hour, within horizontal glass tubes containing culture media. Hodson[5a]), Leonian and Lilly[10a]) used the dry weight method for biotin determinations with the same organism.

This institute had the favour of receiving from prof. Beadle a strain of *Neurospora crassa* Shear and Dodge, an „inositol-less" mutant strain No. 37401 (Beadle)[1]). Since this strain has for some time been used successfully in this laboratory for the determination of biotin in molasses, the details of these investigations are presented here. The growth-rate curves are based on the weights of dried mycelia.

Some strains of *Rhizobium trifolii* were also tested but, for this

organism, the method could not be satisfactorily reproduced. The same was true for *Collybia dryophila* Fr., a biotin-heterotrophic hymenomycete (Lindeberg) [11]).

Methods. The basal medium used was principally the same as that reported by Ryan *et al.* [17]), a modification of Fries' No. 3 medium [3]). It had the following composition:

NH_4 tartrate	5.0 g	B	0.01 mg
NH_4NO_3	1.0 g	Cu	0.10 mg
KH_2PO_4	1.0 g	Fe	0.20 mg
$MgSO_4.7H_2O$	0.5 g	Mn	0.02 mg
NaCl	0.1 g	Mo	0.02 mg
$CaCl_2$	0.1 g	Zn	2.0 mg
Dextrose	10.0 g	Inosit	12.5 mg
		H_2O	1000 ml

The pure biotin added was β-biotin from Hoffman la Roche.

The cultures were made in 100 ml Erlenmeyer flasks (Jena Geräte Glass), treated with chromate-sulphuric acid. Each flask contained 20 ml basal medium. The solution was sterilized by autoclaving at a pressure of 15 lb for 15 minutes. The pH measured at the beginning of the experiments was generally 5.4 and fell at most to 4.6 during the course of the experiments. Inoculations were made by adding 1 ml of conidial suspension to each flask. The conidia, taken from stock cultures grown on a malt extract agar (2.5% malt extract, 1.5% agar), were suspended in sterilized water. The suspension was passed through a sterilized cotton filter in order to remove pieces of mycelium. The conidia were then washed by centrifuging in sterilized water. The number of conidia per ml of suspension was determined in a haematocytometer and adjusted to about 5×10^4 per ml. The growth experiments were carried out at 25°C. Each series comprised 4 parallel cultures. With this density of conidial suspension an incubation period of 5 or 6 days was found to be suitable. The mycelia were removed from the flasks, washed in distilled water, and dried to a constant weight at 102°C.

The water content of the molasses was determined, and varied between 14–24%. Since molasses is considered to contain all its biotin in a water-extractable form (Lampen *et al.*) [8]) the samples of molasses were extracted by autoclaving 10 gr. (dry wt.) of the

material in 1000 ml of water for 10 minutes at a pressure of 15 lb (S n e l l *et al.*) [20]. The pH of the extracts of molasses was generally about 7.5.

Results. As mentioned earlier, R y a n *et al.* [17]) showed, that the rate of growth of the *Neurospora* strain R 977 was a function of the biotin concentration in the range between 0.0128 and 20 mγ per 15 ml of medium. From the shape of their growth-curve, it should be possible to make determinations of a biotin content ranging from about 0.1 to 2.0 mγ per culture. In a preliminary experiment, therefore, biotin concentrations doubled successively from 0.05 mγ to 3,2 mγ per flask were used and measurements of the weight of mycelium were made daily over a period of 2–15 days. In subsequent experiments it was considered sufficient to take only the concentrations in the range between 0.1–1.6 mγ per flask for the standard curve, and an incubation period of 6 days. The variation in the dry weights of mycelia in duplicates of the same series was small. The growth in the controls with no biotin was inconsiderable, generally 0.2 mg in 6 days. An amount of biotin of 0.05 mγ could be measured accurately. However, even though the amount of inoculum remained constant, and the conidia were of the same age, the growth of different series varied a little from one experiment to the other. Presumably these fluctuations were due to the varying vigour of the stock-culture. At the beginning of the investigation, for instance, the shortest test-period that was suitable was that of 6 days (see Table I). Later, when the stock-culture had often been transferred, an incubation time of 5 days was more suitable. In any case, it is obvious that a standard curve must be determined for each experiment.

TABLE I

Biotin content ($m\gamma \times 10^{-3}$) per mg of molasses determined from various amounts after 4, 5 and 6 days incubation.

Amount of Molasses (mg)	Molasses 1			Molasses 2		
	Incubation-time in Days					
	4	5	6	4	5	6
5.0	128	116	120	146	130	134
2.5	120	120	119	129	129	129
1.25	120	128	119	128	122	130

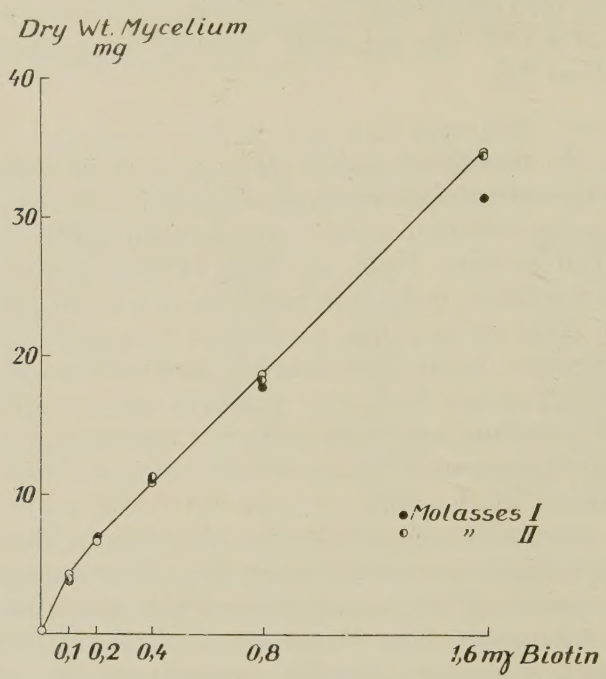

Fig. 1. The growth-rate curve of *Neurospora* with increasing amounts of biotin, and the values of molasses I and II plotted against this curve.

Fig. 1 shows a standard biotin curve from which the biotin contents of two types of molasses were calculated. These values and the dry weights of mycelia are given in Table II. The average biotin-content of molasses I was 124×10^{-3} mγ/mg, that of molasses II, 127×10^{-3}. The experiment was repeated later when the values obtained were 120 and 128, respectively. Determinations of the biotin content of a number of other types of molasses are summarized in Table III. From this table it is obvious that it is possible to determine biotin quantities in the range between 0.10–1.4 mγ. There is good agreement between the values obtained using different amounts of the molasses.

In order to find out whether the molasses extract had any effect on the growth of *Neurospora* other than that which was due to its biotin content, a series of experiments using pure biotin was compared with one in which half the biotin quantity was supplied as pure biotin and the remaining half as a molasses extract (see

TABLE II

The effect of biotin and molasses I and II on the growth of *Neurospora* (6 days incubation)

Biotin		Molasses				
			I		II	
mγ per flask	Dry weight of mycelium (mg)	mg per flask	Dry weight of mycelium (mg)	Biotin found per mg of material ($m\gamma \times 10^{-3}$)	Dry weight of mycelium (mg)	Biotin found per mg of material ($m\gamma \times 10^{-3}$)
0.0	0.2 ± 0.0					
0.1	4.3 ± 0.0	25/32	3.8 ± 0.0		3.9 ± 0.0	
0.2	6.8 ± 0.0	25/16	6.9 ± 0.0	126	7.1 ± 0.1	128
0.4	10.9 ± 0.3	25/8	11.1 ± 0.1	126	11.2 ± 0.3	128
0.8	18.6 ± 0.2	25/4	17.7 ± 0.2	119	18.3 ± 0.3	125
1.6	34.2 ± 0.5	25/2	30.9 ± 0.4		34.1 ± 0.2	
		25/1	51.5 ± 0.7		52.1 ± 1.3	

TABLE III

Biotin content ($m\gamma \times 10^{-3}$) per mg of different molasses types determined from various amounts (5 days incubation)

Molasses Type No.	Amount of molasses (mg)			
	10.0	5.0	2.5	1.25
1		120	119	119
2		134	129	130
3		98	100	100
4		104	100	100
5		98	100	100
6		97	96	98
7		115	—	121
8		125	124	122
9	89	90	93	90
10	108	119	120	120
11	123	123	128	120
12	125	123	126	121
13	121	127	125	120
14	138	139	130	130

R y a n *et al.* [17]· and their assay for *p*-amino-benzoic acid in yeast extract). Molasses I and II were used and the necessary amounts were calculated from the specification of their content given in Table II. The total concentrations of biotin used were the same as in the standard curve, i.e. 0.2, 0.4, 0.8 and 1.6 mγ per flask. The values are given in Table IV and Fig. 2. These agree with those obtained in the previous series and indicate that the growth of *Neurospora* responds only to the biotin fraction of the molasses extract.

TABLE IV

The effect of biotin and biotin + molasses on the growth of *Neurospora* (6 days incubation)

Biotin added per flask, (mγ)	Dry weight of mycelium (mg)		
	Pure biotin	Pure biotin + molasses	
		I	II
0.0	0.2 ± 0.0		
0.2	7.0 ± 0.2	6.4 ± 0.1	6.8 ± 0.1
0.4	11.8 ± 0.2	11.2 ± 0.4	11.8 ± 0.1
0.8	18.8 ± 0.6	19.1 ± 0.5	18.5 ± 0.3
1.6	34.8 ± 0.5	34.1 ± 1.2	33.3 ± 0.6

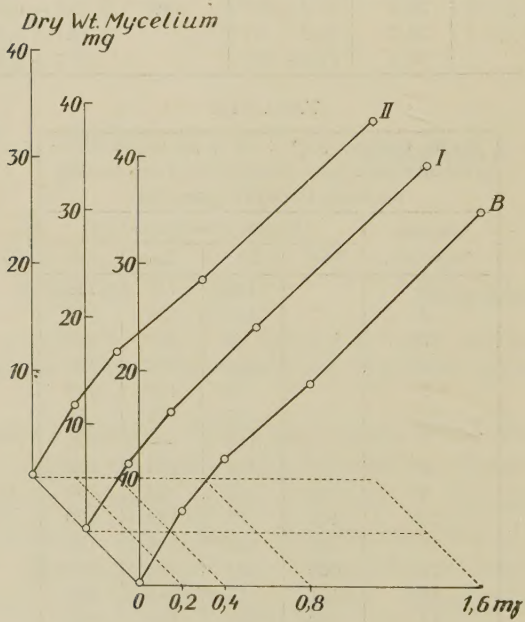

Fig. 2. The effect of biotin and biotin + molasses on the growth of *Neurospora*. Curve B refers to pure biotin; curve I and II, to pure biotin + molasses I and II, respectively.

Summary

The growth responses of *Neurospora crassa* strain No. 37401 to biotin were determined by weighing dried mycelia. It was possible to use a simple synthetic basal medium. Controls with no biotin only gave inconsiderable growths, and the variations within all series were minute. The incubation time was 5 to 6 days. The sensitivity of the assay is indi-

cated by the fact that an increment of 0.05 mγ/20 ml (1 part per 4×10^{11}) could be measured with accuracy. Molasses had no effect on the growth other than that due to its biotin content.

The assay method can be used for biotin quantities within a range of 0.1 — 1.4 mγ per 20 ml. Determinations at various levels within this range showed an average deviation of biotin content per mg of about 2 per cent.

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UTILISATION DES ENGRAIS PHOSPHATÉS PAR LES PLANTES, APRÈS LEUR ABSORPTION DANS LE SOL

par G. BARBIER, J. CHABANNES et A. MARQUIS

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Le pouvoir absorbant du sol à l'égard des phosphates est souvent considéré comme néfaste: il retiendrait sous des formes pratiquement inassimilables la majeure partie des phosphates solubles appliqués, et cela non seulement dans quelques types de sols particuliers, comme les latérites, mais dans la plupart des sols. Les cultures ne récupéreraient en règle générale, pas plus de 20% environ des engrais phosphatés. Cette opinion est basée sur 3 séries de faits:

1°. Les phosphates solubles incorporés dans le sol deviennent avec le temps de plus en plus difficiles à extraire par des réactifs chimiques.

2°. Il a été parfois constaté que l'efficacité des engrais phosphatés s'abaisse fortement au cours de leur vieillissement dans le sol en l'absence de toute végétation (S c a r s e t h et T i d m o r e) [5].

3°. Si l'on compare les quantités de phosphore contenues dans une récolte en présence et en l'absence d'une fumure phosphatée, on constate que la différence est en général petite par rapport à la dose appliquée, 10% environ, souvent moins. L'arrière-effet sur une 2ème culture est encore plus petit, et sur une troisième à peu près nul, de sorte que la somme des effets successifs, et même la limite de cette somme pour une série indéfinie de cultures, apparaît comme très éloignée d'une utilisation totale.

Cette manière de voir, appliquée à la généralité des sols, se heurte à une grave objection. Si les plantes ne pouvaient récupérer, en tout et pour tout, que 1/5ème des phosphates solubles incorporés, il faudrait, pour entretenir la fertilité, fournir au sol cinq fois plus de phosphates que les cultures n'en consomment. Or il semble bien que, dans de très nombreux sols, on puisse maintenir la fertilité

a son niveau actuel en se contentant de restituer les quantités exportées. C'est d'ailleurs ce qu'admettaient les agronomes du siècle dernier; d'après eux, si l'on maintient constante la réserve de phosphore du sol, par apport de phosphates solubles, on maintient cette réserve capable de livrer une quantité constante d'acide phosphorique à une culture déterminée dans des conditions déterminées. Ceci implique que la totalité des phosphates incorporés serait exportable par une succession de cultures suffisamment prolongée. C'est cette contradiction que nous avons essayé de résoudre.

1. *Conservation de l'efficacité des engrais phosphates pendant 16 ans dans un sol de limon.* Gericke [4]) a constaté récemment, dans des sols variés, que l'efficacité des engrais phosphatés se trouve à peine affaiblie après 18 mois de vieillissement en terre nue. Mais il admet qu'elle doit continuer à s'abaisser lentement pendant trés longtemps. Or, nous disposions à Versailles d'un champ d'expériences ayant 16 ans d'existence. Il a été institué en 1928 par Demolon et Burgevin. Il comporte des parcelles recevant chaque année une dose fixe d'un engrais simple, notamment du superphosphate ou des scories. Le sol est constamment maintenu sans végétation, mais reçoit des façons culturales normales.

Les phosphates incorporés ont subi dans ce sol, un limon moyen légèrement acide (pH 6.3 en 1928, s'abaissant à 5,8 en 1942), des transformations profondes. Si l'on porte sur un graphique en abscisses les années (ou ce qui revient au même la quantité totale de P_2O_5 incorporée depuis le début) et en ordonnées le taux de P_2O_5 extractible par des réactifs à action faible (acide acétique dilué notamment) on constate que ce taux augmente de moins en moins vite pendant les premières années, mais qu'il s'accroît ensuite chaque année d'une quantité constante: les diagrammes obtenus commencent par une courbe convexe qui se prolonge par une droite légèrement ascendante. La présence de cette droite ascendante indique que la fixation des phosphates tend vers un équilibre comportant une extractibilité non nulle. Il faut environ 5 ans pour que chaque apport annuel ait terminé son évolution.

Or, dans ce champ d'expériences, les apports d'engrais ont cessé en 1941 (14 apports). Nous disposions donc, en 1944, d'un sol contenant des engrais phosphatés ayant vieilli en moyenne 10 ans environ, la dernière application n'étant pas éloignée du terme de son

évolution. Nous pouvions donc considérer que l'efficacité de ces engrais correspondait sensiblement à l'influence-limite du vieillissement.

Nous avons prélevé en 1944, de la terre de ces parcelles et l'avons utilisée comme engrais phosphaté dans des cultures d'orge en vases; nous avons comparé son action fertilisante à celle d'une même quantité du même engrais appliquée immédiatement avant le semis.

Fumure phosphatée: 0,200 g P_2O_5 pour 10 kg d'un sol brun calcaire contenant 12% $CaCO_3$
Poids de matière sèche récoltée

	Sans engrais phosphaté	Engrais phosphaté appliqué en nature immédiatement avant le semis	Engrais phosphaté ayant vieilli 10 ans en sol légèrement acide
Superphosphate. .	100	186	184
Scories Thomas . .	100	140	177

Le superphosphate vieilli a exercé sensiblement la même action fertilisante que le superphosphate récent. Les scories vieillies ont été sensiblement plus efficaces que les scories récentes.

Ces résultats, qui confirment pour une longue période ceux de Gericke, ne cadrent pas avec la doctrine de l'inactivation progressive des phosphates par fixation irréversible dans le sol. Si une telle inactivation a lieu, elle ne peut se produire que dans le court intervalle qui sépare l'application de l'engrais et l'enracinement des plantes semées immédiatement après l'engrais.

2. *Absorption élective des engrais phosphatés, par les plantes, et épargne des réserves du sol.* Jusqu'à présent, le rôle propre des engrais phosphatés dans la nutrition des plantes a été apprécié par la méthode indirecte déjà rappelée, qui consiste à comparer les quantités de phosphore contenues dans une récolte en présence et en l'absence d'engrais. Cette méthode repose sur l'hypothèse que les plantes emprunteraient aux réserves du sol la même quantité de phosphore dans les deux cas. Mais les apparences seraient les mêmes si les plantes prélevaient électivement l'engrais et laissaient les réserves.

Dans des expériences de cultures de maïs sur solutions en vases

à deux compartiments (avec division du faisceau radiculaire) une première série de plantes a reçu une solution nutritive riche en phosphates (36 ou 18 mg P_2O_5 par litre) dans l'un des compartiments, et une solution pauvre en phosphates dans l'autre compartiment (1 mg, 8 P_2O_5 par litre) Une autre série a reçu la même solution pauvre dans les 2 compartiments. Nous avons constaté que la solution pauvre associée à la solution riche fournit moins de phosphates aux maïs que la solution pauvre associée à la solution pauvre.

Concentration de la solution pauvre après 24 h (concentration initiale = 1,8 mg P_2O_5 par litre)	
Solution pauvre associée à solution pauvre	Solution pauvre associée à solution riche (18 mg)
0,17	0,70

La suralimentation d'une partie des racines a réduit l'alimentation des autres. La même expérience a été répétée dans de la terre, en vases et en plein champ. Du superphosphate a été mélangé à une petite portion du sol (1/16ème dans le cas des vases) et le mélange a été réparti dans des cylindres en toile métallique, disséminés dans la masse du sol non additionnée d'engrais phosphatés.

Après culture, on a déterminé par l'analyse la perte de phosphore total subie par la terre des cylindres. En vases, une orge a prélevé environ 60% de l'acide phosphorique ajouté dans les cylindres, et 50% en plein champ.

Par ailleurs, si l'on fait le bilan complet du phosphore dans ces expériences, on peut calculer la quantité réellement prélevée sur les réserves primitives du sol, puisque l'on connait la quantité totale consommée par les plantes (par l'analyse de la récolte) et la quantité réellement prélevée sur l'engrais. On constate ainsi que les réserves du sol fournissent en présence d'engrais moins de phosphates aux cultures qu'en l'absence d'engrais. Cet effet d'épargne des réserves du sol a atteint 60% en vases, et 40% au champ, représentant 22 kg P_2O_5 par hectare.

On s'explique ainsi pourquoi un apport d'engrais phosphaté augmente généralement peu la consommation de phosphates par les cultures. Ces faits expliquent également pourquoi l'efficacité des

engrais phosphatés disparaît rapidement en terre cultivée, sans s'opposer à sa conservation en terre nue.

3. *Adsorption des phosphates à la surface des argiles, notamment en liaison avec les cations alcalino-terreux* [3]). Un fait demeure étonnant: c'est la conservation de l'efficacité des engrais phosphatés en sol nu, malgré l'abaissement progressif de leur solubilité dans certains réactifs chimiques. C'est pourquoi nous avons recherché si l'absorption des phosphates dans le sol ne présente pas quelque particularité susceptible d'expliquer cette anomalie.

A. Demolon et G. Barbier [2]) ont constaté que le calcium des argiles intervient directement dans la rétention des phosphates, indépendamment de toute précipitation de phosphates calciques proprement dits. Les faits suivants confirment l'existence de composés d'adsorption comprenant argile, ions calcium, ions phosphoriques, nettement distincts des phosphates calciques, et précisent les conditions de leur formation.

1°. Différentes argiles (un sous-sol de limon, une kaolinite et une montmorillonite), retiennent à l'état calcique, beaucoup plus de phosphates qu'à l'état potassique, même lorsque la concentration initiale de la solution en phosphates est notablement inférieure au seuil de précipitation des phosphates calciques dans les conditions des essais.

2°. Si l'on fait varier la concentration en phosphates, on obtient des courbes caractéristiques des phénomènes d'adsorption typiques: la quantité de phosphates absorbée devient proportionnellement d'autant plus petite que la concentration dans le solvant est plus grande, aussi bien pour les argiles Ca que pour les argiles K.

3°. Entre pH 6 et 7 environ, l'alcalinisation par addition de chaux entrave la rétention des phosphates par les argiles calciques, c'est-à-dire favorise le maintien des phosphates en solution.

4°. Les équilibres s'établissent rapidement: la quantité supplémentaire de phosphates retenue par les argiles Ca atteint pratiquement un maximum après $^1/_4$ d'heure d'agitation des suspensions aqueuses. La progression ultérieure de l'absorption des phosphates ne dépend pas de la nature du cation échangeable.

5°. En l'absence de sel floculant, les argiles Ca n'adsorbent qu'une petite quantité de phosphates; l'adsorption augmente très rapidement par addition de traces d'un sel floculant (chlorure ou sulfate de

Ca) jusqu'a un maximum correspondant à la floculation complète de l'argile (environ 2 N/100), comme si les ions phosphoriques ne pouvaient être retenus qu'entre deux surfaces soudées par la floculation.

Pour préciser l'importance quantitative de ces composés d'adsorption par liaison calcique, dans le sol en place décrit plus haut (qui contient des engrais phosphatés ayant vieilli 10 ans), nous sommes partis de l'hypothèse que les acides dilués, qui détachent facilement le calcium échangeable, doivent séparer en un temps court, les ions phosphoriques ainsi retenus. Effectivement, les acides dilués à froid (acétique ou sulfurique) à pH 3.0, extraient une fraction importante des phosphates appliqués, et cela d'une façon presque explosive, comme on peut s'enrendre compte en percolant une mince couche de terre sur le vide, de manière que la terre soit traversée en quelques secondes. Par 3 ou 4 extractions acides de courte durée, on récupère environ $^1/_3$ des engrais appliqués. Un autre $^1/_3$ se sépare instantanément si l'on reprend le résidu par un alcali trés dilué (2 N/1000). Le phénomène de rétrogradation ou de „fixation" des phosphates dans le sol étudié, paraît consister essentiellement en une évolution limitée de formes facilement extractibles par les acides dilués vers des formes facilement extractibles par les alcalis dilués *) (probablement comme conséquence d'une adsorption lente par les hydroxydes ferriques qui recouvrent d'un mince enduit la surface des particules minérales). Or le passage de la Ière à la 2ème forme ne paraît pas entraver notablement l'alimentation phosphorique des plantes dans le sol étudié, car les scories de déphosphoration, dont l'acide phosphorique est resté beaucoup plus facilement extractible par les acides dilués que celui du superphosphate, ne manifestent pas, après 10 ans, une efficacité supérieure à celle du superphosphate. Ceci peut expliquer pourquoi une perte de solubilité *dans les réactifs acides,* n'entraîne pas nécessairement une perte d'efficacité.

Conclusion. Il ne convient pas d'exagérer les inconvénients du pouvoir absorbant des sols à l'égard des phosphates. La présente étude ne résout pas la question de savoir si une fraction des phosphates solubles incorporés dans le sol peut y former des composés complètement et définitivement inertes. Mais elle montre que cette fraction, si elle n'est pas nulle, est assez petite, du moins dans les

* Voir sur le même sujet l'article de A. Dean [1]).

sols qui ne donnent pas lieu à une inactivation des phosphates par vieillissement.

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NEUERE BEOBACHTUNGEN ÜBER DIE URSACHEN DER DÖRRFLECKENKRANKHEIT BEIM HAFER

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1. *Einleitung.* Wie ein roter Faden können im agrikulturchemischen Schrifttum Angaben über Schädigungen infolge starker Kalkdüngung festgestellt werden. Wohl einen der ältesten Hinweise finden wir nach Ehrenberg [5]) bei J. N. von Schwerz [21]), der 1823 Wachstumsschäden auf humusarmen Böden infolge kräftiger Aetzkalkdüngung erwähnt. Seither wurden für ähnliche Wachstumsstörungen verschiedene Erklärungsversuche mitgeteilt, die sich meistens auf entsprechende Untersuchungen stützen können. Nach den Symptomen der „Kalkschäden" darf vielfach auf eine Störung im Wasserhaushalt der Pflanzen geschlossen werden, während weiter aus entsprechenden zusätzlichen Düngungen abgeleitet werden musste, dass beim Überschreiten einer bestimmten Kalksättigung im Boden die Löslichkeit und Aufnahmebereitschaft einiger Nährstoffe herabgesetzt werden, so vor allem von Kali (Ehrenberg [5a]), Gisiger) [7]), Magnesium (Loew) [13]), Phosphorsäure (Liechti–Truninger) [23]) Eisen (Molz) [16]) und Bor (Brandenburg) [2]).

Hudig [11]) zeigte in seiner Arbeit über eine „eigentümliche Bodenkrankheit", die er mit der von Clausen [3]) beschriebenen *Dörrfleckenkrankheit* des Hafers als identisch betrachtet, dass diese Krankheit ebenfalls eine starke Abhängigkeit von der Kalkversorgung der Böden und ihrer Reaktion aufweist. Dabei können unter Umständen an sich ganz geringfügige Veränderungen der Reaktion nach der alkalischen Seite durch Anwendung physiologisch alkalischer Dünger das Auftreten der Krankheit stark begünstigen. Umgekehrt wirken physiologisch saure Dünger, vor allem schwefelsaures Ammoniak, in der Richtung der Krankheitsverhütung. Ganz

geringe Gleichgewichtsstörungen können also die Krankheit auslösen oder auch verhindern.

Angeregt durch Arbeiten von Loew über die stimulierende Wirkung der Mangansalze auf das Wachstum der Pflanzen, prüfte Hudig in seinen Versuchen über die Dörrfleckenkrankheit auch verschiedene Mangansalze. Diese ergaben als Überraschung das Resultat, dass es möglich ist, durch Mangandüngung die Dörrfleckenkrankheit zu verhüten. Seit dieser Entdeckung wurde der Praxis empfohlen, durch angemessene Mangananwendung selbst den stark zur Dörrfleckenkrankheit neigenden Hafer vor Erkrankung zu schützen.

2. *Von den unmittelbaren Ursachen der Dörrfleckenkrankheit.* Im Laufe der Jahre wurden verschiedene Hypothesen über die unmittelbaren Ursachen der Dörrfleckenkrankheit aufgestellt. Für diese Frage scheint uns die Empfehlung Hudigs [11]) in ihrer Konsequenz, die Mangansalze nicht zu früh anzuwenden, wegweisend, muss doch daraus geschlossen werden, dass die an sich erwiesenermassen günstig wirkenden löslichen Mangansalze im dörrfleckenkranken Boden zunehmend festgelegt werden können. Darnach wäre die Dörrfleckenkrankheit in erster Linie auf das Unlöslichwerden und die zu geringe Aufnehmbarkeit des Mangans in den kranken Böden zurückzuführen. Diese Auffassung wurde später von verschiedenen Forschern geteilt, so auch von Samuel und Piper [20]), die aber weiter dem Verhältnis von Kali zu Kalk grosse Bedeutung zuerkennen. Nach ihnen scheint es, als ob zu extreme Quotienten K : Ca an sich, auch ohne offenbaren Mangan-mangel, die Krankheitsursache sein können. Wir werden später auf diese Feststellungen zurückkommen.

Nach Lundegardhs [14]) Versuchen spielt die Art und Menge des Humus keine wesentliche Rolle für das Auftreten der Dörrfleckenkrankheit, wie dies Hudig annimmt. Nicht ohne weiteres ist aber die Feststellung begreiflich, dass die Krankheit im Gegensatz zur Ansicht Arrhenius [1]) durch zu hohe Kalziumkonzentration nicht besonders begünstigt wird, während sie aber recht häufig bei Kalziummangel im Blattgewebe auftritt. Scheinbare Widersprüche ergeben sich auch aus der Untersuchung von gesunden und kranken Pflanzenorganen auf ihren Mangangehalt. Während Samuel und Piper glauben, als Schwellenwert zwischen

gesund und krank 12–14 p.p.m. (0.0012–0.0014%) Mangan angeben zu können, fand Lundegardh [14]) vollkommen gesunde Pflanzen mit einem Mangangehalt von nur 0.0005% Mn., während umgekehrt in Blättern von Hafer auf typisch kranken Böden 0.0012–0.042%! Mangan ermittelt werden konnte.

Es ist offenbar weniger der absolute Gehalt als das Verhältnis des aufgenommenen Mangans zu andern Nährstoffen im weitesten Sinne des Wortes (vergleiche auch Hiltner) [10]) von Wichtigkeit. Die kurative Wirkung der Mangananwendung dürfte ebenfalls in dieser Richtung eine Erklärung finden, wobei sich der günstige Effekt sogar in der Grenzschicht Wurzel-Boden, also in der Wurzelzone, abspielen kann und in der Zusammensetzung der oberirdischen Organe als Folge der Mangandüngung nicht unbedingt eine Gehaltsänderung eintreten muss. Dabei darf aber nicht übersehen werden, dass die Entwicklung der Krankheit unterbunden werden kann, wenn im Moment des Auftretens der ersten Krankheitsflecken auf die Pflanzen Mangansulfat gesprüht wird, wobei u.U. mit einer wesentlich geringeren Manganmenge auszukommen ist, als wenn dieses bei der Saat auf oder gar in den Boden gebracht wird.

Im Vergleich zu den bisherigen Feststellungen brachten die Versuche von Popp [19]) und Mitarbeitern und von Maschhaupt [15]) insofern eine Überraschung, als dabei grosse Mengen Kalk in Form von CaO gegeben als Heilmittel gegen die Dörrfleckenkrankheit erkannt wurde. Wir finden allerdings bei Lundegardh schon früher gleich gerichtete Beobachtungen, die auf ein Minimum der Löslichkeitskurve für das Bodenmangan im neutralen pH-Bereich zurückgeführt wurden. Das Mangan würde also im sauren und stark alkalischen Boden bessere Löslichkeit aufweisen als auf neutralem Boden. Diese Angaben werden durch die Mangangehalte der Pflanzen von sauren und neutralen bis schwach alkalischen Böden insoweit bestätigt, als wohl eine Abnahme des Gehaltes verzeichnet wird; dagegen konnte bisher mit Ausnahme der Befunde Lundegardh's keine gesicherte Zunahme des Mangangehaltes der Pflanzen von Böden mit neutraler bis schwach alkalischer Reaktion nach stark alkalisch festgestellt werden. In unsern früheren Versuchen über den gegenseitigen Einfluss von Kali und Kalk konnte auf den gut mit Kalk gesättigten Böden im Gegensatz zu den stark sauren der Mangangehalt der

Pflanzen (Lein, Rettich, Senf) 25–30 mal kleiner gefunden werden (Gisiger)[9]).

3. *Widersprechende eigene Versuche.* *a.* Warum neue Untersuchungen über das Auftreten der Dörrfleckenkrankheit? Seit der Entdeckung Hudig's fanden die Mangansalze ausgedehnte Anwendung zur Verhütung der Dörrfleckenkrankheit. In der Schweiz litt vor allem die Staatsdomäne Witzwil seit den 1920er Jahren stark unter dieser Krankheit *). Auch hier wurde nach Angaben der Direktion regelmässig und mit Erfolg das Getreide mit Mangansulfat behandelt. Während des vergangenen Krieges konnte die notwendige Menge Mangansulfat nicht mehr beschafft werden, durch diesen Mangel war auf grossen Flächen der erwähnten Domäne der Getreidebau in Frage gestellt. So ergab sich aus der Praxis die grundsätzliche aber ebenso dringende Frage, ob durch eine andere Massnahme das Auftreten der Dörrfleckenkrankheit verhütet werden kann.

b. Versuche auf Neulandböden. Bevor wir auf die Besprechung unserer Untersuchungen über die Dörrfleckenkrankheit eingehen, müssen wir zum bessern Verständnis unserer späteren Ausführungen einige Versuche streifen, die im Zusammenhang mit den Anbauschwierigkeiten auf Neuland durchgeführt wurden. Dabei handelte es sich meistens um Böden, die relativ kalkreich waren. Im Wallis tritt noch grosse Trockenheit bei relativ hohem Grundwasserstand hinzu, was zu Ausblühungen und Salzbildung in den obersten Schichten führte. Auf den Böden der Linthebene konnte von einer Salzbildung nicht gesprochen werden, trotzdem waren hier ähnliche Beobachtungen über Wachstumsschäden zu machen wie im Wallis. (jährliche Niederschlagsmenge im mittleren Wallis: 60 cm bei hohem Grundwasserstand; in der Linthebene 120 cm; die Anbauschäden wurden durch die relativ langen Schönwetter- bis Trockenperioden der letzten Jahre verschärft). Die Versuche zur Bekämpfung der Neulandkrankheit sollten in erster Linie zeigen, dass die Böden nicht wegen einseitigem

*) Die Staatsdomäne verfügt über schwach sauren bis neutralen Moorboden, auf dem der Kehricht der Stadt Bern nach längerem Kompostieren verwendet wird. Das Auftreten der Dörrfleckenkrankheit (Kehrichtkrankheit) wird durch den kalkhaltigen Kehricht bedingt, möglicherweise verschärft durch dessen Borgehalt.

Mangel von Spurenelementen unbefriedigendes Wachstum ermöglichten, sondern typisch *überschusskrank* waren, wobei dieser Überschuss durch Auswaschung bezw. Absenkung des Grundwasserstandes entfernt werden kann.

Im Prinzip wurden deshalb die verschiedenen Böden je in 2 Gefässreihen eingefüllt, von denen die eine während 14 Tagen täglich 2 mal durchgespült wurde. Nach Ablauf dieser Zeit erhielten beide Gefässreihen gleiche Düngung und wurden mit den „kalkempfindlichen" Pflanzen Lein und Senf angebaut. Gestützt auf frühere Angaben über die Festlegung von Bor und Phosphorsäure auf gut mit Kalk gesättigten Böden wurde als Differenzdüngung zu den Hauptnährstoffen noch Bor und Phosphorsäure verabfolgt.

Die beiden Abbildungen 1 und 2 sind typisch für alle Versuche und zeigen folgendes:

1. Auf den kranken, unausgewaschenen Böden können mit einer üblichen Volldüngung weder Lein noch Senf zu normaler Entwicklung gebracht werden (Gefässe 1).

2. Nach Zugabe von Bor zeigen die Versuchspflanzen auf nicht ausgewaschenem Boden überraschend günstiges Wachstum (Gefässe 2).

3. Die zusätzliche Phosphorsäuredüngung blieb fast wirkungslos (Gefässe 3 und 6).

4. Durch *kurzfristiges Auswaschen* wird die kranke Erde so verändert, dass die Versuchspflanzen bei normaler Volldüngung mit Phosphorsäure, Stickstoff und Kali praktisch normales Wachstum zeigen (Gefäss 4 im Vergleich zu 1).

5. Auf dem ausgewaschenen Boden vermochte die zusätzliche Borsalzdüngung eine gerade merkbare Wachstumsbegünstigung zu bringen (Gefässe 5 im Vergleich zu 4).

Da durch die relativ kurz dauernde Auswaschung der Bauschchemismus der Böden kaum merkbar verändert werden konnte, ist anzunehmen, dass der Entfernung der Kationen geringe unmittelbare Bedeutung zukommt, sie ist mehr mittelbar darin zu suchen, dass die im Zusammenhang mit der Hydrolyse des Bikarbonates entstehenden OH-Ionen entfernt wurden, die bekanntlich stark quellend auf das Pflanzenplasma wirken; mit dieser Ansicht erklärt sich auch ganz zwangslos die überzeugend günstige Wirkung des Bors, das hinsichtlich Quellung den OH-Ionen entgegengesetzt wirkt, soll es doch Gelatine dehydratisieren.

Abb. 1. Wachstum von Senf auf schwerem Neulandboden.

1 2 1 2
4 5 4 5
nicht ausgewaschen
vor dem Anpflanzen ausgewaschen
e — mit — ohne — mit Bor
ohne — mit — ohne — mit Bor
PNK — Düngung überall gleich
Lein Senf
Lein Senf

Abb. 2. Wachstum von Lein und Senf auf „überschusskrankem" Boden.

c. Versuch mit ausgewaschenem, dörrfleckenkrankem Boden. In Anlehnung an die eben geschilderten Versuche mit sehr günstigen Ergebnissen und gestützt auf die zitierten Ansichten Hudigs, wonach die Dörrfleckenkrankheit vor allem auf gut gekalkten Böden auftritt, setzten wir mit dörrfleckenkrankem Boden eine Doppelserie an, wobei ein Teil der Gefässe wiederum während 2 Wochen ausgewaschen wurde, um die Entfernung von überschüssigem, leichtlöslichem und vor allem basisch wirkendem Kalk zu erreichen.

Bei 2facher Wiederholung wurden die ausgewaschenen und nicht ausgewaschenen Gefässe nach Zugabe der gleichen Düngung mit Hafer angesät. Nach dem Versuchsprotokoll zeigte der Hafer im 4-Blattstadium *auf den ausgewaschenen Gefässen* auffallend weniger gesundes Wachstum und stärkeren Befall mit Dörrflecken als auf der unbehandelten Erde. Trotzdem der Boden an und für sich kalireich war, brachte die Kalidüngung eine auffallende Wachstumsbegünstigung, während namentlich im Wachstumsbild die zusätzliche Magnesium- und Mangandüngung nicht sehr zu überzeugen vermochte. Aus dem Versuchsprotokoll verdient weiter festgehalten zu werden, dass die Manganreihe eher stärkeren Befall zeigt als die Magnesiumreihe. Wenn auch das Bild der Vegetation mit der Entwicklung eine Verschiebung erfuhr und so die Erntegewichte nicht als ausgesprochen typisch zu betrachten sind, möchten wir dieselben doch in der folgenden Tabelle wiedergeben:

TABELLE I

Korn (K) — und Stroh (St) — Erträge auf verschieden behandeltem dörrfleckenkrankem Boden *)

Düngung	PN		PNK		PNK + $MgSO_4$		PNK + $MnSO_4$		PNK + $CuSO_4$	
	K	St	K	St	K	St	K	St	K	St
nicht ausgewaschene Reihen	27,8	36,8	30,1	41,2	33,8	43,2	32,1	43,0	31,9	41,2
ausgewaschene Reihen . . .	9,1	30,5	19,7	34,2	23,0	36,0	20,5	36,4	—	—

Die Ertragszahlen sind immer noch charakteristisch und zeigen vor allem zwischen den beiden Reihen die nachteilige Wirkung des Aus-

*) Dörrfleckenkranker Boden will heissen, dass auf diesem Boden das Getreide dörrfleckenkrank wird.

waschens und innerhalb der Reihen die vorteilhafte Wirkung der zusätzlichen Kali- und Magnesiadüngung, während weder das angewandte Mangan- noch Kupfersulfat zu überzeugen vermochten.

Wohl wurden dem Boden auf 3,5 kg durch die Auswaschung 7,42 g CaO, 0,76 g MgO, 0,46 g K_2O, 0,21 g Stickstoff und 2,1 mg Bor entzogen. Chlor und Sulfate waren im Durchflusswasser aber nur in Spuren vorhanden, sodass anzunehmen war, die Auslaugung erfolgte über Bikarbonat. Da dieses in der Hydrolyse OH-Ionen bildet, darf gefolgert werden, dass der ausgewaschene Boden geringeren OH-Ionen-Druck zeigte als der unbehandelte Boden. Umgekehrt wagten wir daraus zu schliessen, dass die Zufuhr von OH-Ionen durch Düngung mit Hydratkalk oder anderen basisch wirkenden Salzen eine Wachstumsbegünstigung gebracht hätte (für diese Hypothese zeugt auch die Beobachtung, wonach die Magnesiumdüngung eher günstiger gewirkt hat als die Mangandüngung) und im Gegensatz dazu eine Bordüngung das Krankheitsbild verschlimmert hätte. Dieser letzte Schluss schien uns umso berechtigter, als nach bisher unveröffentlichten früheren Versuchen in Oerlikon eine zusätzliche Bordüngung auf dörrfleckenkrankem Boden zu Hafer und Sommerroggen z.T. ganz beachtliche Mindererträge brachte.

TABELLE II

Trockensubstanz-Erträge in g je 3 Gefässe von Hafer und Roggen auf dörrfleckenkrankem Boden

Düngung	Hafer Wassersättigung des Bodens in % der Kapazität		Sommerroggen Wassersättigung des Bodens in % der Kapazität	
	60	80	60	80
PNK	79,0	57,5	96,8	93,0
PNK + $MnSO_4$	99,0	63,5	101,0	87,0
PNK + Bor	84,0	39,5	88,0	59,0
Ertragsdifferenz durch Bor	+ 5,0	—18,0	—8,8	—34,0

Weiter überrascht an diesen Versuchen die wenig vorteilhafte Wirkung der höhern Wassersättigung, eine Feststellung, auf die hier nicht näher eingetreten wird.

4. *Versuche mit steigenden Borgaben auf gesundem Moorboden.* Um zu prüfen, ob durch Bordüngung allein auf gesundem Moorboden die

Dörrfleckenkrankheit des Hafers verursacht werden kann, wurden Gefässversuche angelegt, wobei zu normaler Volldüngung von 0–400 kg/ha steigende Borsäuregaben verabfolgt wurden.

Der Versuchsboden: Zur allgemeinen Charakteristik des Versuchsbodens mögen die folgenden Angaben dienen.
Humusgehalt des trockenen Bodens: 74,4%; pH H_2O : 6,3 pH KCl : 6,0
Aschengehalt des trockenen Bodens: 25,6%
Karbonate: fehlen.
Leichtlösliche Phosphorsäure: in gesättigtem CO_2 Wasser: Testzahl 6,0
lactatlöslich: mg/100 g 17,5
Leichtlösliches Kali: Bikarbonatlöslich: 3,8 mg/100 g
Neutralisationskraft
S = 151 maeq/100 g trockenen Bodens, entsprechend . . . 4,23% CaO
Hydrolytische Acidität
$y_1 = 85{,}5$ cc $\frac{n}{10}$ Na OH/50 g Boden, entsprechend:
$T - S_{bis\ pH\ 7} = 25{,}7$ maeq/100 g trockenen Bodens, entsprechend 0,72% CaO
$T - S_{bis\ pH\ 7{,}5} = 34{,}20$ maeq/100 g Boden, entsprechend . . . 0,96% CaO
bis pH 8,0 : 42,75 maeq/100 g Boden, entsprechend . . . . . 1,2 % CaO
bis pH 8,5 : 55,6 „ „ „ „ . . . . 1,56% CaO
Absorptionskraft
$T_{pH\ 7} = 176{,}7$ maeq/100 g Boden, entsprechend . . . . . 4,95% CaO
berechnet nach *Kappen*
Austauschacidität
$y_1 = 2{,}15$ cc $\frac{n}{10}$ Na OH.

Über die Entwicklung des Hafers gibt das Versuchtsprotokoll die folgende Auskunft: Der Hafer wurde während der ganzen Versuchsdauer *nicht dörrfleckenkrank*, dagegen zeigten die Blätter mit steigender Borsäuregabe zunehmend dürre Spitzen. (nicht verwechseln mit der Spitzendürre = Dörrfleckenkrankheit). Damit geht eine kaum merkbare Wachstumshemmung parallel, die erst bei 200 kg Borsäure auffallend stark ist und bei 400 kg Borsäure/ha das Eingehen der Pflanzen bedingte. Als auffallendes Merkmal verdient noch hervorgehoben zu werden, dass die grosse Mehrzahl der obersten Blätter die Spitze eingeknickt hatten, wie es aus den Abbildungen 3 u. 4. zu erkennen ist, eine Erscheinung, die nicht selten auch an Freilandpflanzen beobachtet werden kann und auf eine Störung im Wasserhaushalt zurückzuführen ist. Entsprechend den während des Wachstums gemachten Aufzeichnungen konnten erst bei übermässigen Borsäuregaben Ertragseinbussen festgestellt wer-

Typische Störung der Blattentwicklung des Hafers
bei hohen Borsäuregaben.

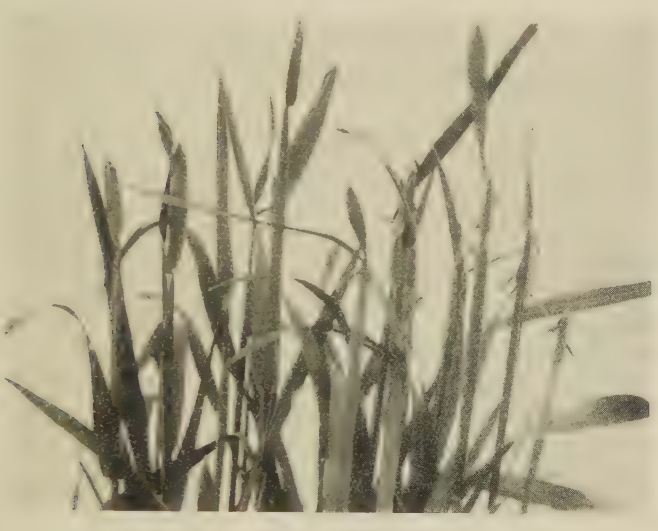


Abb. 3. Oberste Pflanzenpartien eines Gefässes.

Abb. 4. Einzelne Blätter.

den, wie dies aus der folgenden Tabelle zu erkennen ist, in welcher zur bessern Illustration der Wirkung steigender Gaben Borsäure auch die Erträge eines Versuchs auf mineralischem Boden unseres Versuchsareals mit Hafer und die entsprechenden Ertragszahlen mit Gerste angegeben werden.

TABELLE III

Körner- und Stroh-Ertrag von gesunden Böden bei steigender Borsäuredüngung, Versuchspflanzen Hafer und Gerste

Borsäuredüngung in kg/ha		0	6.25	12.5	25.0	50.0	100	200	400
Trockensubstanz-Ertrag in Gramm je Gefäss									
Hafer									
Moorboden	Körner	69,9	70,7	69,7	68,5	65,9	63,7	40,0	kein
	Stroh	78,4	79,7	80,6	80,2	79,9	86,7	70,9	Wachstum
Mineralboden	Körner	68,9	67,1	68,3	67,0	66,8	48,7	1,0	kein
	Stroh	76,6	73,2	76,1	74,4	79,5	66,8	13,6	Wachstum
Gerste									
Moorboden	Körner	72,8	70,6	54,7	67,8	66,8	57,9	33,6	1,2
	Stroh	75,7	77,6	77,1	75,4	75,3	65,8	59,0	12,1
Mineralboden	Körner	58,9	56,8	52,5	55,9	47,1	25,1	0	kein
	Stroh	62,5	60,9	61,1	58,0	55,2	48,4	12,1	Wachstum

Damit war für uns ausreichend erwiesen, dass durch Bordüngung allein auf gesundem Moorboden die Dörrfleckenkrankheit nicht verursacht werden kann, weshalb die unter b.1 gemachte, allerdings mit wenig Aussicht für Erfolg sprechende Schlussfolgerung als weitere Arbeitshypothese gewählt wurde. Wenn auch bei Hafer als Versuchspflanze mit den steigenden Borgaben bis 25 kg Borsäure/ha nicht von einer Ertragsabnahme gesprochen werden kann, ist eine solche bei der Gerste schon bei den kleinen Gaben deutlich erkennbar.

5. *Tastversuche mit dörrfleckenkranker Erde und steigender Kalkdüngung in Form von Branntkalk* (*CaO*). Trotz den eindeutig ungünstigen Resultaten, die auf ausgewaschenem, dörrfleckenkrankem Boden erhalten wurden, schien uns der Schluss sehr gewagt, dass von massiver Verschiebung der Reaktion des Bodens nach der alkalischen Seite eine Gesundung zu erwarten ist, diese Skepsis war

umso berechtigter, als der für die Versuche verwendete kranke Boden rund 4% kohlensauren Kalk enthielt. Es wurde denn auch nur ein Tastversuch in gut 1 kg Erde fassenden Tontöpfen angelegt. Bei gleicher Grunddüngung erhielten die Gefässe steigende Mengen Kalk in Form von Branntkalk, es wurden die folgenden Stufen gewählt:

CaO je kg Erde: 0; 0,25; 0.5; 1.0; 2.0; 3.0; 4.0; 6.0; 8.0 u. 10.0 g.

Als Versuchspflanze wurde Hafer am 20. April eingesät. Er zeigte auf den Gefässen ohne Kalk bis und mit 1.0 g CaO am 28. Mai die ersten Merkmale der Dörrfleckenkrankheit. Später trat eine gewisse Verschiebung der Krankheit in der Versuchsreihe ein, die Pflanzen auf „ohne und mit kleinen Mengen Kalk" waren eher gesünder als die Pflanzen auf dem Boden mit mittleren Kalkgaben. Lange waren die Pflanzen mit „6 g Kalk" am gesündesten und üppigsten, später wurden diese aber auch leicht krank. Wenige Tage vor dem Auftreten der ersten Dörrflecken ergab die Reaktionsmessung die in der

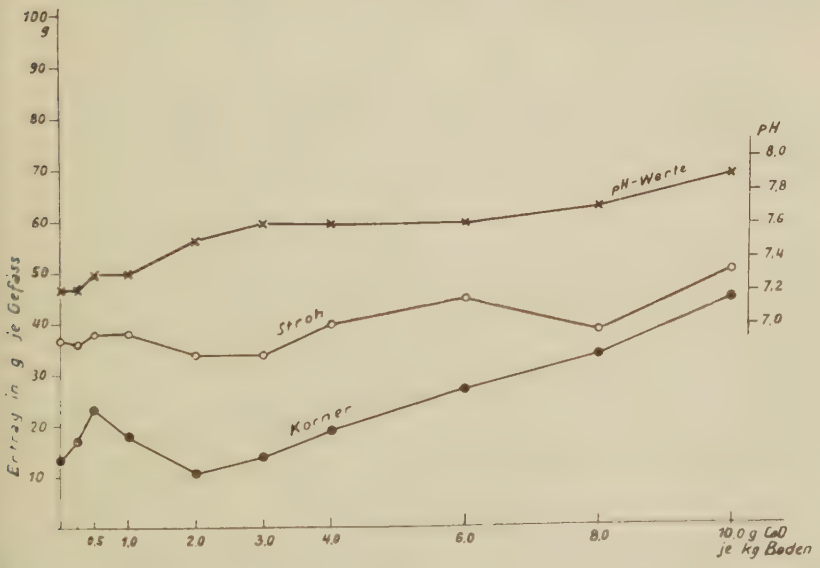


Abb. 5. Einfluss steigender Aetzkalkgaben zu dörrfleckenkrankem Boden auf Körner- und Strohertrag des Hafers und die pH-Zahl des Bodens.

Abb. 5 aufgetragenen pH-Werte. Diese Ergebnisse mit den Aufzeichnungen des Versuchsprotokolls verglichen, zeigen, dass die

Krankheit im pH-Bereich von 7,2–7,6 am stärksten auftritt und bei höheren pH-Werten im Boden wieder zurückgeht. Leider waren uns damals die in der Einleitung zitierten Versuche von Popp [15]) und Maschhaupt [18]) nicht bekannt, weshalb für uns die günstige Wirkung übergrosser Ätzkalkgaben als Widerspruch und Überraschung neu war. Diese war dennoch umso glaubhafter, als sie die Arbeitshypothese als zutreffend und richtig bestätigte. Obwohl der Versuch nur einfach, also ohne Wiederholungen angesetzt wurde und der Einzelwert nicht sehr gesichert erscheint, halten wir die ganze Reihe wegen ihrer eindeutigen Tendenz namentlich im Körnerertrag als typisch.

Die getrennte Untersuchung der Blätter und des Strohs des reifen Hafers ergab für die Mineralstoffe Phosphorsäure, Kali, Kalk, Bor und Mangan keine nennenswerten Unterschiede für die verschiedenen Kalkgaben. In 100 g Blatt-Trockensubstanz wurde 1–2 mg Mn gefunden, die Pflanzen auf „ohne Kalk" enthielten 3,2 mg, die Schwankungen scheinen aber rein zufällig und die Gehaltszahlen an Mangan verraten keinen sichern Zusammenhang mit der Kalkdüngung.

6. *Der Einfluss steigender Kalk- und Borgaben auf gesundem Moorboden auf die Dörrfleckenkrankheit.* Zu den Versuchen des Jahres 1945 mit steigenden Borsäuregaben (Abschnitt 4) wurden 4 verschiedene Pflanzen verwendet, sodass für jedes Verfahren bei 3-facher Wiederholung 12 Gefässe zur Verfügung standen. Die Erde dieser Gefässe wurde im Herbst des gleichen Jahres zusammen gemischt und separat aufbewahrt. Es ergaben sich somit 8 Erdposten mit 0–400 kg Borsäure pro Hektare, entsprechend 0–1280 mg Borsäure je Gefäss. Mit dieser so vorbehandelten Erde wurde im Frühjahr 1946 ein Versuch mit steigenden Kalkgaben angelegt, und zwar mit 0, 3, 6 und 12 g CaO je kg Erde. Aus dem Versuchsprotokoll verdienen die folgenden Beobachtungen erwähnt zu werden:

Die Hafereinsaat erfolgte am 26. März.

23. April: Mit steigender Borgabe zeigen die Pflanzen zunehmend dürre Blattspitzen, und zwar schon deutlich erkennbar bei 6 kg Borsäure/ha. Die Pflanzen scheinen von der *Kalkdüngung* zu *profitieren.*

24. April: Ohne Bor sind alle Pflanzen gesund. Von 6,25–50 kg Borsäure pro ha weisen alle Gefässe mit 3 g CaO/kg Boden dörrfleckenkranke Pflanzen auf, nicht aber auf 100–400 kg/ha Borsäure.

1. Mai: Die Beobachtungen lassen sich am besten im folgenden Schema zusammenfassen:

TABELLE IV

Borsäuregabe in kg/je ha	Kalkgabe in g/je kg Boden			
	0	3	6	12
0	gesund	krank	gesund	gesund
6,25	,,	,,	schwach krank	,,
12,5	,,	stark krank	krank	,,
25	,,	stark krank	krank	schwach krank
50	,,	stark krank	krank	schwach krank
100	,,	schwach krank	gesund	gesund
200 }	Bor-	zunehmende Gesundheit, keine Dörrfleckenkrankht. →		
400 }	schäden	abnehmend dürre Blattspitzen (von Borüberschuss)		

Vergl. Abb. 6, 7, 8 und 9.

Nach den späteren Aufzeichnungen traten noch Verschiebungen im Krankheitsbild ein. Als das Wesentlichste seien davon festgehalten:

1. schwach saurer Moorboden wird durch Zufuhr steigender Ätzkalkgaben dörrfleckenkrank, um bei ganz grossen Gaben wieder gesund zu werden.

2. Das Auftreten der Dörrfleckenkrankheit wird durch Borbeidüngung bis zu gut doppelt starker Gabe der üblichen Düngung auffallend begünstigt; bei übergrosser Bordüngung konnte in unseren Versuchen die Dörrfleckenkrankheit durch steigende Ätzkalkdüngung nicht erzeugt werden.

3. Bei 25 kg und 50 kg Borsäure pro Hektare konnte selbst bei der höchsten im Versuch angewandten Ätzkalkgabe die Dörrfleckenkrankheit nicht verhütet werden.

Die nach Körner und Stroh getrennten Erträge der Tabelle V vermitteln ein gutes Bild und sind zugleich eine Bestätigung der Wachstumsbeobachtungen.

Die Ergebnisse unserer Versuche stimmen nur teilweise mit den Angaben von E. Reeve [19]) überein, wonach die schädliche Borwirkung mit zunehmender Kalziumversorgung abnimmt, zeigen wir doch in der Tabelle V von der Spalte 0 Kalk zu 3 g Kalk für alle Borgaben eine sehr starke Ertragsabnahme: dagegen darf in Übereinstimmung mit S. Pontailler [17]) festgehalten werden, dass ein Jahr nach der Düngung eine stärker ins Gewicht fallende Borschädigung auf sauren Böden nach unseren Versuchen erst bei beachtlich hohen Borgaben eintritt, dabei sei ausdrücklich betont,

Einfluss steigender Kalk- und Borgaben auf gesundem Moorboden zu Hafer. (Borwirkung 2tes Jahr nach der Düngung).

Ohne Kalk.

Die steigende Borgabe hat keinen auf dem Bilde sichtbaren Einfluss auf das Wachstum des Hafers (vergl. Tab. 4).

Abb. 6.

3 g Kalk je kg Boden.

Mit steigender Borgabe ist vorerst eine deutliche Zunahme der Dörrfleckenkrankheit zu erkennen, die bei den hohen Gaben wieder weniger oder gar nicht auftritt.

Abb. 7.

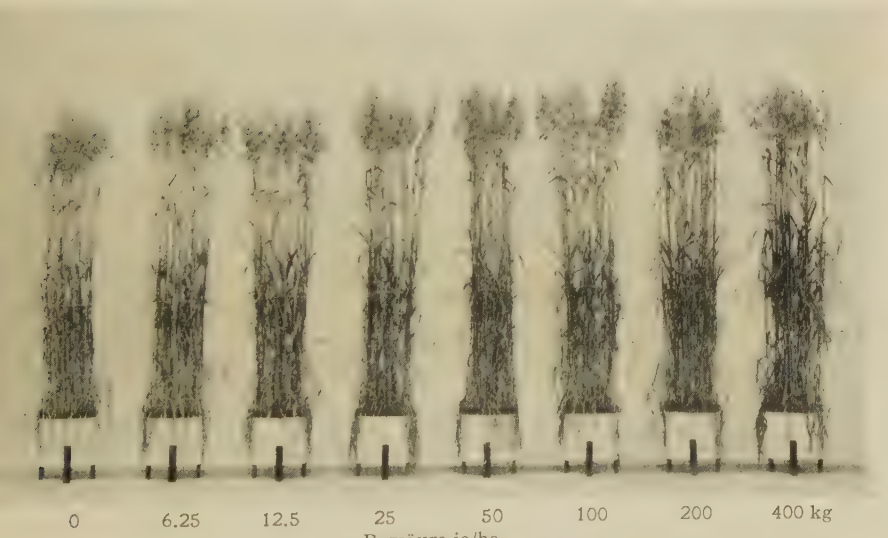
Einfluss steigender Kalk- und Borgaben auf gesundem Moorboden zu Hafer. (Borwirkung 2tes Jahr nach der Düngung).

6 g Kalk je kg Boden.

Hier ist der Einfluss steigender Borsäuregaben auf die Gesundheit des Hafers deutlich zu erkennen; zuerst Zunahme und bei grossen Gaben wieder Abnahme der Krankheit.

Abb. 8.

12 g Kalk je kg Boden.



Bei dieser hohen Kalkgebe hatte die Bordüngung keinen massgebenden Einfluss auf die Dörrfleckenkrankheit, sie trat aber bei den Borsäuregaben 25 bezw. 50 kg/ha auf.

Abb. 9.

TABELLE V

Körner- und Stroherträge von Hafer in g Trockensubst. je Gefäss auf schwach saurem Moorboden bei steigender Aetzkalk- und Bordüngung

Borsäure mg je Gefäss (bezw. kg/ha)	Ätzkalkgabe in CaO je kg Boden							
	0		3 g		6 g		12 g	
	Körner	Stroh	Körner	Stroh	Körner	Stroh	Körner	Stroh
0	51,4	69,1	21,7	64,7	45,8	68,8	55,9	81,2
20 (6,25)	54,4	75,7	10,2	57,7	37,1	74,1	56,2	83,9
40 (12,5)	51,6	77,9	1,9	47,2	15,0	75,9	54,8	86,1
80 (25)	56,8	78,9	2,7	43,0	4,2	62,6	37,5	87,1
160 (50)	58,5	80,9	3,1	49,0	15,3	81,7	49,3	86,8
320 (100)	54,5	75,7	27,0	65,7	47,7	70,7	55,3	80,2
640 (200)	47,0	68,8	31,9	64,4	45,8	69,5	52,5	76,9
1280 (400)	50,3	82,8	36,8	84,9	29,4	96,6	47,7	95,0

dass in unserem Versuch nur Hafer geprüft wurde. Es ist aber anzunehmen, dass die verschiedenen Pflanzen ganz ungleich auf zusätzliche Bordüngung reagieren, worauf vor allem auch F. M. Eaton[4]) hinweist (vergl. Absch. 4).

Während der Durchführung unserer Versuche mit hohen Ätzkalkgaben kamen uns die Beobachtungen von Popp, Contzen und Gericke[18]) und Maschhaupt[15]) zu Gesicht, nach welchen durch Anwendung hoher Ätzkalkgaben die Dörrfleckenkrankheit ebenfalls zum Verschwinden gebracht werden konnte. Popp enthält sich, eine Erklärung für die qualitativ ungleiche Wirkung kleiner und grosser Ätzkalkgaben zu geben, Maschhaupt stellt dafür die Möglichkeit zur Diskussion, mit der auch Storck[22]) rechnet, dass bei sehr hohen Kalkgaben mit einer teilweisen Sterilisation des Bodens gerechnet werden müsse, woraus der Hafer Vorteil schöpfe. Noch fehlten uns bisher entsprechende Untersuchungen, um zu dieser Frage eindeutig Stellung zu beziehen, doch glauben wir, in den schon früher erwähnten Feststellungen Lundegardh's über die Löslichkeit des Mangans die einfachste Erklärung zu finden. Darnach ist anzunehmen, dass im

schwach alkalischen Boden das Mangan weniger löslich ist als bei stärker alkalischen und unter sauren Bedingungen, wie dies in der Abb. 10 von L u n d e g a r d h und in Übereinstimmung mit

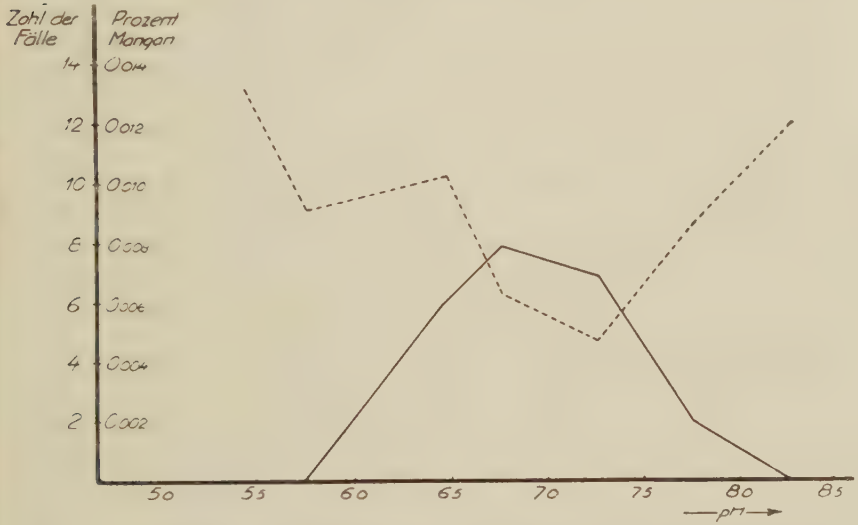


Abb. 10. Graphische Darstellung der Schwankung des Mn-Gehaltes der Böden und des Auftretens der Dörrfleckenkrankheit mit dem pH-Wert des Bodens (nach L u n d e g a r d h)

unseren Befunden über den Mangangehalt des Haferstrohs aus dem zur Diskussion stehenden Versuch zum Ausdruck kommt. Auf die Wiedergabe aller Analysen glauben wir an dieser Stelle verzichten zu dürfen, zeigen doch die Mangangehalte für das Stroh der Verfahren 0–320 mg Borsäure je Gefäss (0–100 kg Borsäure/ha) mit steigender Ätzkalkgabe gleiches Verhalten wie dies in der Tabelle VI zum Ausdruck kommt.

TABELLE VI

Ätzkalkgabe in g je kg Boden	Mangangehalt in mg % von Haferstroh bei einer Borsäuredüngung in mg je Gefäss von				
	0	20	320	640	1280
0	4,0	4,0	4,4	5,0	4,3
3	0,7	1,0	1,3	0,4	4,4
6	0,8	0,8	0,9	0,9	1,9
12	1,3	1,5	1,5	1,3	2,1

Diese Zahlen scheinen in der Grössenordnung den Befund von Sa-

muel und Piper zu bestätigen, wonach als Schwellenwert für das Auftreten der Dörrfleckenkrankheit 1,2–1,4 mg% Mangan zu betrachten sind (vergleiche Tabelle V).

(Der Feststellung über die erhöhte Manganmobilisation bei stärker alkalischer Reaktion steht die Auffassung Russells entgegen, wonach das Mangan des Bodens im pH-Bereich von 7,0–9,0 unlöslich sein soll).

Nach umfassenden Untersuchungen von Gerretsen[6]) ist die erhöhte Manganfestlegung im schwach sauren bis schwach alkalischen pH-Bereich nicht nur rein chemisch, sondern im wesentlichen mittelbar durch Mikroorganismen bedingt, denen weiter für das Auftreten der Dörrfleckenkrankheit massgebende Bedeutung zukommt, indem sie nur ungenügend mit Mangan versorgte Wurzelspitzen befallen und zersetzen können. (Schlaffheit und braune Verfärbung der Wurzelenden). In Übereinstimmung mit andern Forschern kann Gerretsen weiter zeigen, dass Manganmangel allein noch nicht zu ausgesprochener Dörrfleckenkrankheit führt, sondern nur die Disposition für den Befall der Wurzeln schafft. So wird wahrscheinlich gemacht, dass die Dörrfleckenkrankheit sekundär durch Mikroorganismen auf *disponierten Pflanzen* bedingt wird. (Parallele zum Auftreten der Herz- und Trockenfäule der Rüben).

Wir sind uns durchaus bewusst, dass die wiedergegebenen Analysenzahlen (Tab. VI) mit gewissen Fehlern behaftet sind. So mag es auffallen, dass mit 640 mg Borsäure bei 3 g Kalk der tiefste Manganwert gefunden wurde, trotzdem für dieses Düngungsverfahren deutlich gesünderes Wachstum feststellbar war. Vergleichen wir aber alle Spalten mit steigender Borsäuregabe, dann kann man sich des Eindrucks nicht erwehren, dass mit zunehmender Borsäuredüngung eher eine Zunahme des Mangangehaltes parallel geht; die Zunahme scheint für die 1280 mg Borsäuregabe gesichert. So möchten wir wenn auch mit gewisser Reserve den Schluss wagen, dass *bei hoher Borsäuregabe die Manganaufnahme beim Hafer etwas begünstigt und damit das Auftreten der Dörrfleckenkrankheit* zurückgedrängt wird. Interessanterweise kann bei den kleineren Borsäuregaben kein Einfluss weder auf Mangangehalt noch auf den Borgehalt gefunden werden.

7. *Der Einfluss einseitig wechselnder Kali- und Kalkverhältnisse*

der Bodenlösung auf die Dörrfleckenkrankheit. Verschiedene Beobachtungen brachten uns zur Ansicht, dass nebst der OH-Ionen-Konzentration den Kalziumionen einerseits und dem Verhältnis Ca : K eine wesentliche Bedeutung zukomme. Um zur Diskussion dieser Frage einen experimentellen Beitrag leisten zu können, legten wir die folgenden extremen Versuche an.

Zu gesundem Moorboden wurden im Prinzip und pro Gefäss die folgenden Düngungen gegeben:

1,5 g Phosphorsäure, 0,7 g Stickstoff, 1 g Kali als Grunddüngung.

Zusätzliche Versuchsdüngungen:

5 g Kalilauge ohne bezw. mit 15 g, 30 g und 45 g CaO je Gefäss
10 g „ „ „ „ 15 g, 30 g „ 45 g „ „ „
15 g „ „ „ „ 15 g, 30 g „ 45 g „ „ „

Eine kalkfreie Laugenserie wurde nur mit gesunder Moorerde angelegt, während in einer zweiten Serie ohne Kalk zur Moorerde je Gefäss 100 g Sphagnumtorf (holländ. Torf) zugesetzt wurden. Da Laugenschädigungen zu befürchten waren, wurden die Gefässe schon sofort nach der Ansaat auf die volle Wasserkapazität gebracht und Tag für Tag so gegossen, dass Wasser abtropfte; das durchgeflossene Wasser wurde regelmässig zurückgegeben.

Aus dem Versuchsprotokoll möchten wir hier nur einige Hauptpunkte herausgreifen.

10. April: Anlage des Versuches.

5. Mai: Die Pflanzen zeigen noch durchgehend ausgeglichene Gesundheit, die Pflanzen mit der höchsten Kalilauge- und den beiden höchsten Aetzkalk-Gaben sind etwas zurück; mit 10 g KOH und 30 g und 45 g CaO und 15 g KOH und 15 g, 30 g und 45 g CaO zeigen die Haferblätter längs den Nerven Chlorophyllanhäufungen, die Blätter scheinen streifenweise getigert.

9. Mai: Die ersten Dörrflecken erscheinen auf 10 und 15 g KOH der Serie ohne Torf. Von diesem Datum bis Ende Mai nahm die Krankheit zu, ein ungefähres Bild kann aus den Gesundheitsnoten erhalten werden, die am 29.5. aufgenommen wurden (Tabelle VII), wobei 5 keine Dörrfleckenkrankheit angibt und 1 den stärksten Befall.

(Vergl. Abb. 11, 12 und 13).

Als Ergänzung zu diesen Angaben ist noch zu erwähnen, dass die Pflanzen der Gefässe mit nur PN-Düngung deutliche wenn auch nur schwache Kalimangel-Erscheinungen zeigten. Von den in der Tabelle VII wiedergegebenen Aufzeichnungen verdienen die folgenden Erwähnung:

1. Die Dörrfleckenkrankheit tritt sowohl bei schwach saurer

Einfluss steigender Mengen Kalilauge und Aetzkalk
zu gesundem Moorboden.

Abb. 11.

Abb. 12.

Einfluss steigender Mengen Kalilauge und Aetzkalk zu gesundem Moorboden.

Abb. 13.

TABELLE VII

Zusatz in g je Gefäss		PN	PNK + Kalilauge				
Torf	CaO		0	5 g	10 g	15 g	
0	0	5 6,9	5 6,7	3 7,0	1 7,3	1,5 7,7	Gesundheitsnote pH-Werte
100	0	—	5 6,4	4 6,6	3 7,3	2 7,7	Gesundheitsnote pH-Werte
100	15	—	—	3 8,1	4¾ 7,9	5 7,8	Gesundheitsnote pH-Werte
100	30	—	—	4,5 8,3	5 8,2	5 7,9	Gesundheitsnote pH-Werte
100	45	—	—	5 8,2	5 8,4	5 8,1	Gesundheitsnote pH-Werte

(pH 6,6) als auch bei stark alkalischer (pH-Werte 7,7–8,4) Reaktion des Bodenwassers ein, wenn in diesem viel Kali vorhanden ist;

2. Die unter 1 gemachte Feststellung ist aber nur bedingt zutreffend, was an der Reihe 100 g Torf mit 15 g CaO und steigenden Kaligaben erkannt wird, erhielten doch die Pflanzen

mit 5 g Kalilauge die Gesundheitsnote 3
„ 10 g „ „ „ $4^3/_4$
„ 15 g „ „ „ 5.

Die gleiche Andeutung kann auch in der Reihe 30 g Kalk festgestellt werden, während die Reihe 45 g Kalk ganz gesund ist.

3. Auf den ersten Blick mag es scheinen, dass der OH-Konzentration, besonders bei hohen pH-Werten, für das Auftreten der Dörrfleckenkrankheit eine wenig bestimmende Rolle zukomme. Im ganzen Versuch aber zeigt sich eine eindeutige Abhängigkeit zwischen der Bodenreaktion und dem Auftreten der Dörrfleckenkrankheit.

4. In den Kalkreihen wirken die mit steigenden Kaligaben abnehmenden pH-Werte des Durchfluss- bezw. Bodenwassers als Überraschung. Diese Feststellung trat in allen 3 Reihen deutlich in Erscheinung, weshalb sie als wirklich bestehend zu betrachten ist. Da das Durchflusswasser mit zunehmender Kaligabe allerdings durch Humusstoffe stark dunkel gefärbt war, ist die Frage berechtigt, ob dadurch die Chinhydronelektrode ungünstig beeinflusst wurde. Es ist eine solche Annahme abzulehnen, weil die Humussubstanzen wohl reduzierend wirken würden und dadurch das Verhältnis (C-Chinon): (C-Hydrochinon) kleiner 1 würde, womit ein scheinbares Akalischerwerden der Lösung zusammenhinge. (G i s i g e r) [8]).

5. Die dunkle Farbe bezw. die gelösten Humusstoffe des Durchflusswassers scheinen ohne Einfluss auf das Auftreten der Krankheit zu sein.

6. Ganz sicher kann auf gesundem Moorboden versuchsmässig nicht nur durch Kalkzufuhr, sondern auch durch Kaligaben in Form von Lauge die Dörrfleckenkrankheit verursacht werden. Die angewandten Laugenmengen allein reichten nichts aus, um den gesunden Boden auf krank und weiter wieder auf gesund umzustimmen. (Vergl. zu dieser Frage Punkt 2 oben).

Die Zusammensetzung des Bodenwassers. Um Einblick in die Zusammensetzung des Wassers im Versuchsboden und insbesondere Aufschluss über das Verhältnis von CaO : K_2O zu erhalten, wurden

alle Gefässe unmittelbar nach der Ernte gleichmässig durchgespült und das durchgeflossene Wasser mehrmals aufgegossen und so ergänzt, dass überall 250 cc abflossen. Dieses Wasser wurde hernach auf Reaktion und Gehalt an Kali und Kalk untersucht und auf Chlor geprüft. Dieses konnte nur in Spuren nachgewiesen werden. Die erhaltenen Ergebnisse sind in der folgenden Übersicht (Tabelle VIII) mit den Ertragsangaben zusammengestellt.

Aus diesen Angaben ergibt sich in der Detailbetrachtung und im Vergleich zur Tabelle VII folgendes: Auf dem nur mit Phosphorsäure und Stickstoff gedüngten Boden konnte keine Vollernte erzielt werden, die Pflanzen zeigten Kalimangelzeichen, trotzdem in der Bodenlösung 0.24 mg Kali je 100 cc nachzuweisen waren. Durch die Beidüngung von 1 g Kali pro Gefäss trat eine beachtliche Ertragssteigerung ein, nach Aberntung wies das Wasser ungefähr doppelt so hohen Kaligehalt auf als auf ungedüngt (ohne Kali).

Reihe 1. Durch den Zusatz von Kalilauge wurde das Verhältnis von Kalk zu Kali gegenüber der Normaldüngung massiv verändert, und zwar von ca. 100 bei PN und 60 bei PNK auf 1 : 1 bei 5 g, 1 : 2 bei 10 g und 1 : 6 bei 15 g Kalilauge. Mit der steigenden Kalidüngung nahm der Kalkgehalt des Wassers nicht nur relativ, sondern auch absolut ab, was eine bekannte Erscheinung ist. (G i s i g e r)[9]). Durch die erwähnte Verschiebung des Kalk-Kali-Verhältnisses im Zusammenhang mit der steigenden Kalilauge-Düngung stieg der pH-Wert der Lösung von 6.7 nur auf 7.0, was leicht zur Annahme veranlasst, dass die in den Ertragszahlen sich spiegelnde[15]) Wachstumsschädigung in erster Linie auf das veränderte Kalk : Kali-Verhältnis zurückzuführen sei (vergleiche Zahlen 2 mit 3,4 und 5), eine Ansicht, die sich aber nach spätern Untersuchungen nicht halten lässt.

Reihe 2. In der Reihe mit 100 g Torf ohne Kalk wurden leicht tiefere Kalkgehalte festgestellt als in der Reihe 1, was mit der Kalkarmut des zugesetzten Torfs ausreichend erklärt sein mag. Bei normaler Versuchsdüngung wurde eine Vollernte an Körnern und Stroh erhalten, die durch 5 g Kalilauge eine nur unwesentliche Depression erfuhr. Es ist aber schwer zu entscheiden, ob diese nur geringe Ertragseinbusse dem noch sicher über 1 (nahezu 2 : 1) liegenden Kalk-Kali-Verhältnis oder der leicht tieferen pH-Zahl gegenüber bei 3 zu verdanken ist. Es scheint im Vergleich der Zahlen 7, 8 und 9 um den Neutralpunkt für das Auftreten der Dörrflecken-

krankheit von wesentlicher Bedeutung zu sein, ob das Kalk-Kali-Verhältnis der Bodenlösung ca. 2 : 1 oder aber umgekehrt 1 : 2 bezw. gar 1 : 4 ist; von grösserer unmittelbarer Wirkung ist aber möglicherweise die gleichzeitige Änderung des pH-Wertes von 6.6 auf 6.9 und 7.1.

TABELLE VIII

pH-Wert und Gehalt des Durchflusswassers an Kalk und Kali (mg/100 cc) und der Ertrag an Trockensubstanz g/Gefäss getrennt nach Körner und Stroh

Nummer der Tab. Reihe	Zusatz in g je Gefäss		PN	Grunddüngung PNK, dazu g K_2O als Kalilauge				
	Torf	CaO		0	5	10	15	
1	0	0	6.2[1]**	6.4 [2])	6.7 [3])	6.8 [4])	7.0 [5])	pH
			25.1	24.8	25.6	22.3	16.0	CaO
			0.24	0.44	22.4	42.0	100.0	K_2O
			32.3	55.7	39.9	2.5	0.6	Körner
			69.3	84.4	65.5	37.5	28.8	Stroh
2	100	0	—	6.6 [6])	6.6 [7])	6.9 [8])	7.1 [9])	pH
				16.7	19.5	17.5	17.5	CaO
				0.44	11.6	30.0	78.0	K_2O
				55.7	52.1	33.1	6.5	Körner
				89.7	86.4	66.9	47.0	Stroh
3	100	15	—	—	7.7 [10])	8.1 [11])	8.1 [12])	pH
					42.4	36.5	45.2	CaO
					21.2	6.4 *)	6.8 *)	K_2O
					18.0	55.0	55.4	Körner
					68.4	84.7	88.7	Stroh
4	100	30	—	—	7.8 [13])	8.1 [14])	8.0 [15])	pH
					45.7	39.7	33.0	CaO
					34.0	38.0	32.0	K_2O
					44.2	57.0	54.4	Körner
					76.6	89.3	85.9	Stroh
5	100	45	—	—	7.8 [16])	8.0 [17])	8.0 [18])	pH
					17.9	19.6	20.1	CaO
					48.0	50.0	52.0	K_2O
					48.8	51.6	48.6	Körner
					82.1	84.1	81.6	Stroh

*) Bei Wiederholung der Analyse wurden die gleichen Werte getunden, doch scheint ein grober Fehler vorzuliegen, einzig durch den Kalientzug der guten Erträge lassen sich die tiefen Gehalte nicht erklären.

**) Nummern des Verfahrens.

Reihen 3, 4, und 5. Leider lassen sich die Zahlen dieser Reihen nur mit Vorbehalt vergleichen; vor allem scheinen die Kaligehaltszahlen 11 und 12 mit einem Fehler behaftet zu sein.

Auffallen mag, dass mit steigender *Ätzkalkdüngung* keine Veränderung der Reaktion des Bodenwassers, eine solche aber mit der steigenden *Kaligabe* festzustellen ist, im Gegensatz zu den Messungen während der Vegetationszeit. Extreme pH-Werte, wie sie nach der Düngung hätten erwartet werden dürfen, sind nicht aufgetreten, wobei anzunehmen ist, dass in erster Linie Humusstoffe als starke Puffer wirkten. (Das Wasser wurde während der Versuchsdauer immer wieder auf die Gefässe gegossen, mit fortschreitender Zeit trat eine Aufhellung ein, die durch Abnahme der gelösten Humusstoffe zu erklären ist). Überaus komplizierte Verhältnisse zeigen sich im Gehalt des Durchflusswassers an Kalk und Kali, die erneut zeigen, wie schwer es ist, mit einem so komplexen System, wie es der Moorboden ist, eindeutige und klare Ergebnisse zu erhalten. Es mag dabei auch der Umstand mitgewirkt haben, dass nach dem Ausreifen des Hafers während einiger Zeit die Erde nicht begossen wurde und das Austrocknen irreversible Festlegung verursachte.

Wesentlich ist in diesen Reihen die Tatsache, dass vom Verfahren 10 mit 5 g Kalilauge und 15 g Ätzkalk eine Wachstumsbegünstigung sowohl durch höhere Kalilaugen-Zufuhr als auch durch erhöhte Kalkgabe erzielt wird, wobei die erhöhte Kaligabe den grösseren Effekt zeigt. Nach den verwendeten Zusätzen beurteilt, darf also als sicherstehend angenommen werden, dass eine *namhafte Gesundung des Bodens durch einseitige Zufuhr von Kalilauge und Ätzkalk erzielt wird*, eine Feststellung, die gegen einen Einfluss des Kalk-Kali-Verhältnisses spricht, die aber unbedingt für den unmittelbar günstigen Einfluss der mit den Laugen zugeführten OH-Ionen spricht. In diesem Zusammenhang sei vor allem auf die Werte der Verfahren 7,8 und 9 mit ausgeglichenem CaO-Gehalt bei steigendem Kaligehalt des Durchflusswassers und jenen der Verfahren 16,17 und 18 hingewiesen, die bei ähnlichem Kalkgehalt der Lösung wie die Reihen 7, 8 und 9 mit dem Kaligehalt zwischen 8 und 9 liegen, die aber ganz unbefriedigenden Ertrag brachten.

8. *Versuche mit gesundem Moorboden, der nach der Kalidüngung bezw. Kali- und Kalkdüngung bis zur Chlorfreiheit ausgewaschen*

wurde. Mit dem Ziele, zur Frage des Einflusses des Kalk-Kali-Verhältnisses auf die Dörrfleckenkrankheit des Hafers ein auf breiter Basis stehendes Urteil zu erhalten, wurde gesunder Moorboden in 3-facher Serie mit steigenden Mengen Kali in Form von Kaliumchlorid versetzt und vor dem Anpflanzen mit Hafer bis zur Chlorfreiheit ausgewaschen.

Die Serie 1 blieb ohne Kalk, der Serie 2 wurden vor dem Auswaschen 30 g Kalk in Form von Ätzkalk zugesetzt, während die Serie 3 diese Kalkgabe erst unmittelbar vor dem Anpflanzen erhielt.

Während der Auswaschung wurde das Durchflusswasser portionenweise untersucht. Von den erhaltenen Werten interessieren in diesem Zusammenhang vor allem jene des letzten Durchflusswassers, das einen gewissen Anhaltspunkt über die Mobilität und Menge von Kalk und Kali im Versuchsboden gibt.

In der Abb. 14 ist folgendes zu erkennen:

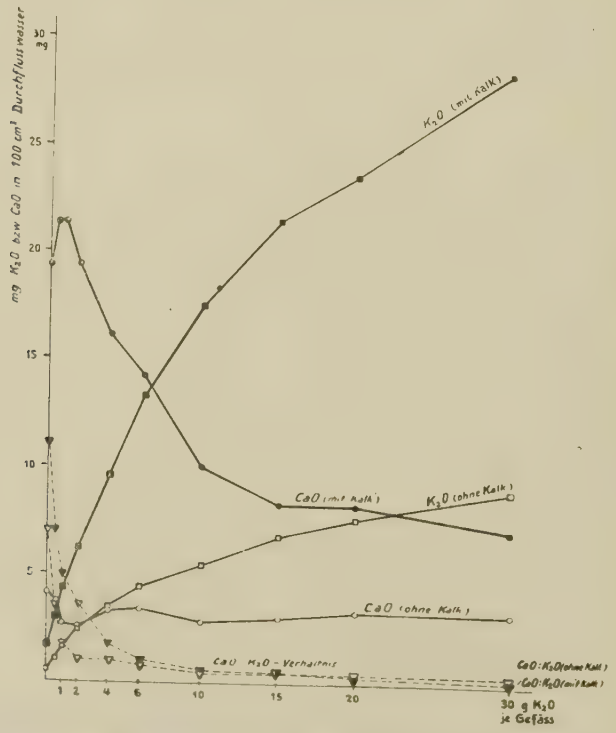


Abb. 14. Kali- und Kalkgehalt im letzten Auswaschwasser (chlorfrei), bei steigenden Kaligaben, mit und ohne Kalk.

Der gekalkte und ungekalkte Boden ohne und mit nur geringer Kalidüngung gibt mehr Kalzium an das Durchflusswasser ab als Kali, wie dies besonders deutlich in der Kurve für das CaO : K_2O-Verhältnis (gewichtsmässig gerechnet) zum Ausdruck kommt, wobei auffällt, dass es von der 6 g Kaligabe an für den un- und gekalkten Boden fast gleich ist. Es ist dies umso auffallender, als bei gleicher Kalidüngung der gekalkte Boden eine wesentlich höhere Kalimobilität aufweist als der ungekalkte Boden. Das aus diesem stammende Durchflusswasser enthält nach gleicher Auswaschintensität nur den 3. Teil des Kali wie aus dem gekalkten Boden, das Verhältnis ist für den wenig und stark mit Kali gedüngten Boden sehr konstant.

Für den Wachstumsversuch standen im Frühjahr 1946 3 verschiedene Serien zur Verfügung, nämlich alle mit der folgenden Kalidüngung, vor dem Auswaschen gegeben:

Kaligabe in g K_2O je Gefäss: 0 0,5 1,0 2,0 4,0 6,0 10,0 15,0 20,0 30,0
Serie I blieb ohne Kalk
Serie II 30 g CaO je Gefäss vor dem Auswaschen
Serie III 30 g CaO je Gefäss nach dem Auswaschen und vor dem Anpflanzen.

Vor dem Anbau wurde überall die gleiche Phosphorsäure- und Stickstoffdüngung gegeben.

Als auffallende Beobachtungen während des Wachstums seien erwähnt, dass in der ohne Kalk gebliebenen Reihe das Wachstum gesünder und üppiger war als auf den beiden Kalkreihen, von welchen die Reihe mit Herbstkalkung und nachheriger Auswaschung auf den Gefässen mit den höhern Kaligaben vorübergehend ziemlich starken Befall mit Dörrfleckenkrankheit zeigte, während die Erkrankung bei Frühjahrskalkung durchgehend so stark auftrat, dass vorzeitig geschnitten werden musste. Es scheint, dass mit 30 g Kalk nicht genügend alkalische Reaktion erzielt wurde, um den Hafer gesund erhalten zu können. Vorstellbar ist aber auch, dass mit der steigenden Kalidüngung in Form von Kaliumchlorid ohne Kalkung im Austauschprozess die Aussengarnitur des Absorptionsschwarmes verdrängt, also durch Kali ersetzt und ausgewaschen wurde, womit eine physiologisch wirksame Mobilisation von Mangan durch günstige und hohe OH-Konzentration rein sterisch verhindert war. Dieser Hinweis zeigt erneut, wie schwer es ist, im Boden klare

Beziehungen einer Abhängigkeit der Dörrfleckenkrankheit vom CaO : K_2O-Verhältnis herauszuschälen.

So betrachtet, ist es nicht auffallend, dass bei Herbstkalkung und nachheriger Auswaschung die Dörrfleckenkrankheit nur bei mittleren und höheren Kaligaben vorübergehend auftrat, nicht aber bei typischem Kalimangel und sehr weitem CaO : K_2O-Verhältnis. Eine Zeitlang war sogar bei den mittleren Kaligaben ein Maximum des Krankheitsbefalles festzustellen. Diese Beobachtungen decken sich mit den Feststellungen von Arrhenius[1]), wonach hohe Kalziumkonzentration nicht unbedingt Anlass zum Auftreten der Krankheit ist, während diese durch Kalziumarmut in den Blättern begünstigt wird. (In unsern Versuchen entsprechen hohe Kaligaben: Kalireichtum und geringer Kalkgehalt in der Bodenlösung).

Im Vergleich mit den Kali- und Kalkgehaltskurven (Abb. 14) des letzten Durchflusswassers unmittelbar vor dem Anpflanzen lassen die Ertragszahlen (Tab. IX) erkennen, dass mit der zunehmenden Kalidüngung eine Ertragszunahme verbunden ist, bis das Verhält-

TABELLE IX

Körner- und Stroherträge des Hafers

Kalidüngung in g K_2O je Gefäss *)	Kalkgaben in g CaO je Gefäss				
	0		30 g vor dem Auswaschen		30 g nach dem Auswaschen
	Körner g	Stroh g	Körner g	Stroh g	Trockensubst. (Grünschnitt) g
0	13,0	37,4	2,3	41,9	15,4
0,5	24,8	44,8	4,1	48,5	10,9
1,0	32,9	48,5	2,6	55,9	10,9
2,0	39,7	49,9	7,7	66,3	10,3
4,0	44,7	54,9	18,6	64,0	9,2
6,0	44,3	54,4	40,3	60,5	6,1
10,0	46,0	56,5	45,0	59,6	9,7
15,0	46,3	57,8	48,7	65,8	6,2
20,0	47,1	60,2	44,6	67,4	5,8
30,0	43,4	60,2	40,8	63,5	5,1

*) Vom gegebenen Düngerkali waren bei der Versuchsanlage noch 70–45% bei der kalkfreien bezw. im Frühjahr gekalkten Serie und 54–42% bei der vor dem Auswaschen gekalkten Serie im Boden. Die tiefere Prozentzahl bezieht sich auf die grössern und die höhere Prozentzahl auf die kleineren Kaligaben. Es stand also für die Vegetation nicht die hier angegebene Kalimenge sondern nur noch ein Teil davon zur Verfügung.

nis CaO : K_2O *) des Wassers sicher unter 1 fällt. Bei noch grösserer Kaligabe fällt das Verhältnis unter 0,5 und bleiben die Erträge konstant, um erst bei der höchsten Kaligabe leicht zu fallen.

9. *Zusammenfassung und Schlussfolgerung.* Nach einem allgemeinen Hinweis auf Wachstumsschäden bei übermässiger Kalkdüngung werden einige unmittelbare Ursachen erwähnt, die für das Auftreten der Dörrfleckenkrankheit am ehesten verantwortlich scheinen. Die bisherigen Versuche weisen eindeutig auf *Mangan-mangel* hin, der bei schwach sauer bis schwach alkalischer Reaktion sich am ungünstigsten auswirkt. Weiter muss stark damit gerechnet werden, dass das CaO : K_2O-Verhältnis für das Auftreten der Dörrfleckenkrankheit eine Rolle spielt. Es hält aber schwer, hierzu eindeutige Versuche beizubringen. Nach den Ergebnissen neuerer systematischer Versuche, veranlasst durch das vorübergehende Fehlen der notwendigen Mangansalzmengen für die Bekämpfung der Dörrfleckenkrankheit, ist folgendes zu erkennen:

1. Auf gut mit Kalk gesättigten, ungenügend durchlässigen oder schlecht drainierten Böden treten Anbauschwierigkeiten auf, die durch Wässern oder Drainage beseitigt werden können, denen aber auch durch Anwendung von Borsalzen entgegengetreten werden kann. (Störung des Nahrstoffgleichgewichtes durch einseitigen Überschuss).

2. Überraschenderweise kann durch kurzes Auswaschen eines dörrfleckenkranken Bodens das Auftreten der Krankheit begünstigt werden, darauf zurückzuführen, dass sich die Entfernung der OH-Ionen (basisch wirkender Salze) und eventuell ungünstig hoher Borgehalt des Bodens als nachteilig auswirken.

3. Auf schwach saurem Moorboden kann durch steigende Borsäuregaben die Dörrfleckenkrankheit bei Hafer nicht erzeugt werden.

4. Auf schwach saurem Moorboden wird durch Ätzkalkgaben, die zur Neutralisation des Bodens gut ausreichen, Dörrfleckenkrankheit des Hafers verursacht, die bei sehr hohen Ätzkalkgaben wieder verschwindet.

*) Weitere Versuche mit Kalziumchlorid und Gips zur Prüfung des Einflusses hoher Ca-Konzentration führten zu keinen auswertbaren Ergebnissen, weil bei Anwendung von Chlorid die hohe Löslichkeit des Salzes zu plasmolytischen Störungen führte und der Gips nicht sehr hohe Kalziumkonzentration zuliess. Ebenfalls plasmolytische Störungen wurden in der Reihe mit steigenden Kaliumchloridgaben beobachtet, wenn vor dem Anpflanzen die Gefässe mit hohen Gaben nicht ausgewaschen wurden.

5. Im zur Dörrfleckenkrankheit neigenden Boden wird diese durch steigende Borsäuremengen stark begünstigt, sie wird aber durch hohe Borsäuremengen, 100–150 kg pro ha, selbst auf dem neutralen bis schwach alkalischen Moorboden verhindert. (Eine Erklärung für diese Erscheinung konnte bisher nicht gefunden werden, das Verhindern der Dörrfleckenkrankheit ist sehr wahrscheinlich auf erhöhte Manganaufnahme zurückzuführen).

6. Extreme Versuche mit steigenden Zusätzen von Kalilauge und Ätzkalk zu schwach saurem, gesundem Moorboden lassen erkennen, dass die Dörrfleckenkrankheit sowohl durch Kalilauge als auch durch Ätzkalkanwendung hervorgerufen werden kann, ebenso kann die Dörrfleckenkrankheit durch einseitig hohe Kalilaugegaben sowohl als auch durch hohe Ätzkalkgaben wieder zum Verschwinden gebracht werden. Daraus ergibt sich der Schluss, dass für das Auftreten der Dörrfleckenkrankhet in erster Linie die Wasserstoff- bezw. Hydroxylionen-Konzentration verantwortlich ist, während in dieser Hinsicht dem Verhältnis $CaO : K_2O$ eine zurücktretende Bedeutung zukommt. (Grössere Manganlöslichkeit im sauren und stark alkalischen Boden als im neutralen-schwach alkalischen Boden, L u n d e g a r d h).

7. Untersuchungen des Durchflusswassers von gesundem, schwach saurem bezw. stark alkalischem Moorboden einerseits und dörrfleckenkranken neutralen bis schwach alkalischen Moorböden anderseits ergaben ebenfalls kein eindeutiges Bild über den Einfluss der $CaO : K_2O$-Verhältnisse des Bodenwassers für das Auftreten der Dörrfleckenkrankheit. (zur weitern Abklärung dieser Frage sind erneut Versuche angelegt).

8. Als praktische Schlussfolgerung ergeben sich aus den bisherigen Beobachtungen und als Nutzanwendungen die folgenden Empfehlungen:

a. Die Anwendung von Mangan (40–160 kg Mangansulfat pro ha) ist nach wie vor das einfachste Mittel für die Bekämpfung der Dörrfleckenkrankheit.

b. In Zeiten des Manganmangels lässt sich der Getreidebau auf dörrfleckenkrankem Boden sichern:

1. durch Anwendung sehr hoher Kalisalzgaben (diese Empfehlung ergab im Feldversuch günstige Resultate).
2. durch die Düngung mit 150–200 kg Borsäure pro ha und
3. durch Zufuhr von 60–100 q Ätzkalk pro ha.

(Auf dem Boden der Staatsdomäne Witzwil konnte das Auftreten der Dörrfleckenkrankheit als Folge der Verwendung von Kehricht verhindert werden, wo grosse Mengen Kehricht verteilt wurden ($^1/_6$–$^1/_3$ der Krumenschicht).

c. Wo der Boden aber durch ständige Zufuhr von Kalk und kalkhaltigen Abfällen (Witzwil verwendet den anfallenden Kehricht der Stadt Bern kompostiert) nur in der Furchenschicht „dörrfleckenkrank" ist, muss unbedingt geprüft werden, ob nicht durch Heraufholen des schwach sauren Moorbodens mit einer ausgesprochenen Tiefenfurche wieder gesunde Verhältnisse geschaffen werden können.

9. Die in der Praxis für die Verhütung der Herz- und Trockenfäule zur Anwendung gelangenden Borsalzgaben können das Auftreten der Dörrfleckenkrankheit begünstigen, es lohnt sich in dieser Hinsicht nochmals zu prüfen, welches die notwendigen und in der minimalen Dosis ausreichenden Bormengen für die Bekämpfung der Herz- und Trockenfäule sind.

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THE INFLUENCE OF MICROORGANISMS ON THE PHOSPHATE INTAKE BY THE PLANT

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Introduction. The way in which insoluble phosphates are absorbed by the plant is still a matter of some doubt. It is generally accepted that the carbonic acid, excreted by the plant roots, is capable of bringing insoluble phosphates into solution.

This conception ultimately rests upon the classic experiments of Liebig[1]) and Sachs[2]), who demonstrated that polished plates of marble and ostheolite were etched by the roots of different plants.

According to Miller[3]), however, there is as yet no positive proof of the solvent action of carbonic acid, excreted by the roots, upon soil minerals.

Stoklasa c.s.[4]) made an extensive study of the microbiological solubilization of insoluble phosphates; in the majority of their experiments, however, they added to the soil 2,5% glucose, which is oxidized to organic acids by different soil microbes. Besides which no comparision was made with sterile cultures, nor were any phosphate determinations made on the plant material.

Consequently no definite conclusions can be drawn with regard to the influence of microorganisms on the phosphate intake by the plant under natural conditions.

Fred and Haas[5]) were the first to show that the etching of marble by the roots of peas was markedly increased in the presence of bacteria. They concluded that the etching was in no case caused by root excretions alone, CO_2 included, but that microbes in the rhizosphere played a very important rôle in this phenomenon.

Starkey[6]) and Gräf[7]) showed that the number of bacteria in the rhizosphere was far greater than the number at some distance

from the roots. Organic substances excreted by roots, such as was shown by Mazé [8]) (glucose by maize roots), Virtanen [9]) (asparagine by the roots of legumes) and many others, as well as the organic material from dead epidermal cells and expelled caliptral cells, are easily attacked by a number of microorganisms and are the primary cause of the microbiological situation in the immediate surroundings of the roots differing widely from that in the soil at some distance from the roots. Thus it is to be expected that in this rhizosphere especially, processes take place, which are of the utmost importance for the intake of nutrients by the plant.

Recently Rosendahl [10]) and Mitchell c.s. [11]) observed a considerable influence of mycorrhizae on the intake of potassium from feldspat, which also holds good in the case of the absorption of phosphorus from insoluble phosphates.

With regard to our crop plants, however, little has been recorded since the publication of Fred and Haas in 1919 and the interesting work of Dustman [13]) in 1925, who studied the rate of excretion of carbon dioxide from the roots of plants, which can grow well on poor land (buckwheat, rye) compared with those which do not thrive at all in such area's (corn, barley etc.).

The point I wish to emphasize is that neither Dustman, nor any of the numerous workers who studied the excretion of carbon dioxide by the roots of plants, used sterile cultures nor did they pay any attention to the question as to whether the carbon dioxide originates directly from the roots of the plants or whether it is indirectly produced by microorganisms in the rhizosphere.

This, however, is of great importance, particularly in the case of newly reclaimed land, where the number of active soil microbes is generally low and where it is to be expected that the rate of microbiological solubilization of different minerals is much slower than in cultivated soils with their rich and varied microbial population.

In order to answer the question, as to the extent of the rôle played by micro-organisms during the phosphate intake by the plant, it is necessary to experiment with different plants and to cultivate them in sterile as well as in infected media. Both the yield and the quantity of the phosphates absorbed by the plants have to be determined.

General outline of the experiments. As a preliminary experiment

a Neubauer test with rye was carried out in quartz sand, both sterile and infected, with tri-calcium phosphate as the source of phosphate.

Since the Neubauer test has been widely used to determine the availabilty of phosphate in the soil, it was expected that it would also indicate any subsequent influence of the microbes on the availability of the phosphate for the rye plants.

Experiments on a larger scale were carried out in Mitscherlich pots containing 4–5 kg sand or soil, mixed with nutrients. All the fertilized pots were sterilized under steampressure at 120° for at least 6 hours; afterwards half of the pots were infected with 1 % of a fertile soil. In order to ensure reliable comparision the other half received 1 % garden soil as well, but before sterilization (except in the first experiment with oats).

Different difficultly soluble phosphates were used, namely tri- and di-calcium phosphate, bonemeal, Moroccan and Algerian phosphate, whilst in some cases potassium phosphate was used as a standard.

In order to eliminate the influence of the nitrifying bacteria a mixture of KNO_3 and $Ca(NO_3)_2$ was used as a source of nitrogen.

The pots were sown with seeds of oats, mustard (*Sinapis alba*) and sunflower (*Helianthus annuus*). The water content of the pots was regularly controlled by weighing, whilst in some of the experiments a device was used, by which the water content of the sand was automatically kept at a constant level, thus greatly improving the sterility of the cultures.

Finally the plants were harvested, the dry weight (at 105° C) and phosphate determined in an average sample of the ground material. In a number of cases the pH in the rhizosphere was determined by means of a platinum needle electrode, covered with quinhydrone.

From the rhizosphere of the infected roots a number of bacteria was isolated, the pure cultures of which were used to infect sterile pots, in order to verify the extent to which they could influence the phosphate intake of the plants.

In order to demonstrate visually the solubilization of phosphates by bacteria in the rhizosphere, oval glass plates, covered with an agar film in which calcium phosphate had been precipitated, were placed aslant in cylindrical culture vessels. In this way the roots were

forced to grow along the glass plates and the solubilizing activity of the microbes could easily be shown.

Methods. The white quartz sand, used for the pot experiments, contained some soda which was removed by washing with dilute acetic acid, rinsing with tap water until there was no acid reaction and finally with distilled water.

The sand, mixed with the nutrients, for the sterile cultures as well as for those to be infected, was brought to 75% of its water capacity, and sterilized at 120° C for at least 6 hours. The seeds were sterilized with germisan in vacuo, the seed coat of the oats, having to be removed and they were then planted under sterile conditions. In order to keep the cultures sterile, the sand was covered with a layer of sterile cork filings mixed with katadyne silver, the latter by its oligodynamic action killing the invading microorganisms [14]).

The phosphate in the plant material was determined by digesting the ground material in the usual way with a mixture of nitric and sulphuric acid (Fleischmann-acid) followed by precipitation of the phosphate with ammonium molybdate.

Experimental. — Neubauer test, with rye, sterile and infected. Small porcelain pots, filled with 40 g of sand and 8 cc H_2O, containing 5 mg P_2O_5 as $Ca_3(PO_4)_2$, were planted with 12 sterilized rye seeds. We used one tenth of the quantities of sand and of seeds as prescribed by Neubauer [15]), in order to minimize the chance of infection.

The seeds were weighed individually on a sensitive spring balance and those of equal weight selected in order that the quantity of P_2O_5 contained in the seeds would be the same for all pots.

The pots were sterilized and half of them were infected with 1 % of a fertile soil .After being kept under a large glass jar for three weeks the plants were harvested, the roots cleaned from the adhering sand, dried and weighed and the P_2O_5 content of the whole plant determined. The number of bacteria finally present in the soil was counted on Waksman's albumine agar. [16]).

The results are summarized in the following table.

The average P_2O_5 content of the original seeds was 3,33 mg; from the $Ca_3(PO_4)_2$ 1.15 mg had been absorbed by the plants under sterile conditions, and this was increased to 1,88 mg after infection, an increase of 63,5 %. Similarly in the culture which had been spontaneously infected, more phosphate has been made available to the plants.

TABLE I

Neubauer test with rye. P_2O_5 content of the young plants after 3 weeks

Treatment	mg P_2O_5 in 12 plants	Mean	Increase in P_2O_5 content after infection in % of sterile	number of bacteria per gram of soil
Soil, without phosphate sterile	3,30			
	3,37	**3,33**	—	0
	3,37			
The same, infected.	3,40			
	3,23	**3,30**	—	11,3 million
	3,26			
Sand with $Ca_3(PO_4)_2$ sterile . .	4,49			
	4,52	**4,48**	—	0
	4,42			
The same, infected accidentally	4,86	**4,86**	+ 33%	1,5 million
The same, infected with 1% garden soil	5,24			
	5,41	**5,18**	+ 63,5%	15 million
	5,03			
	4,96			

These increases which amount to more than 13 and 7 times resp. the mean error of the determinations, appear to be real and indicate that microbes favourably may influence the intake of phosphates by the plant. These experiments, however, were not continued, because the method proved to be unsatisfactory, due to the difficulty in selecting seeds of equal phosphate content and to the high rate of infection when growing 12 seeds in one pot.

First experiment with oats in sterile and infected quartz sand. The Mitscherlich pots each contained 4 kg sand, dressed with 2 g KNO_3, 0,8 g $Ca(NO_3)_2$, 0,08 g $CaCO_3$, 0,08 g $CaSO_4$, 0,05 g $FeCl_3$, and 1 cc of a mixture of minor elements, containing 0,0005 g H_3BO_3, 0000125 g $CuSO_4$, 0,000125 g KI and 0,025 g $MnSO_4$.

0,8 g P_2O_5 was added as $Ca_3(PO_4)_2$, $CaHPO_4$, $Fe_3(PO_4)_2$, and Algerian phosphate respectively.

All the pots were sterilized, and afterwards half of the pots was infected with 1 % of a garden soil*). The experiments were carried

*) In this case, no garden soil had been added to the sterile pots; for reliable comparison it would have been better to add also 1% garden soil to these pots before sterilisation. This has been done in the other experiments; the results, however, proved to be the same.

out in duplicate. The sterilized oat seeds were germinated on nutrient agar to control sterility, and 5 seeds were then planted in each pot. At the end of the experiment the plants from each pot were dried and weighed together, and the total phosphate content determined. The results are summarized in the following table.

TABLE II

Yield and phosphate absorbed by oat plants, grown in sterile and infected quartz sand (5/8–15/10)

Treatment	Total dry weight of 5 plants. (g)	Mean	Increase in yield by infection in % of sterile	Total P_2O_5 absorbed by the plants (mg)	Mean	Increase in P_2O_5 absorbed by the plants, compared with sterile, in %
Blank, without phosphate	2,0			2,0		
	2,0	2,0	—	1,6	1,8	—
Ferrophosphate sterile	2,4			5,8		
	2,5	2,5		6,8	6,3	
Ferrophosphate infected	4,5			12,6		
	4,0	4,3	72	15,2	13,9	120
Algerian phosphate sterile	3,2			3,2		
	1,7	2,5		1,9	2,6	
Algerian phosphate infected	4,8	4,8	92	11,0	11,0	324
	—			—		
$CaHPO_4$ sterile	3,1			25,0		
	3,1	3,1		34,0	28,5	
$CaHPO_4$ infected	8,2			55,0		
	9,5	8,9	187	76,9	66,0	124
$Ca_3(PO_4)_2$ sterile	3,5			42,0		
	3,2	3,4		30,7	36,4	
$Ca_3(PO_4)_2$ infected	12,5			77,5		
	7,0	9,8	188	82,6	80,1	120
Bonemeal sterile	5,2	5,2		42,0	42,0	
	—			—		
Bonemeal infected	11,3			63,5		
	13,5	12,4	138	86,9	75,2	79

Fig. 1A. The beneficial influence of infection on the intake of phosphorus from insoluble phosphates by oat plants. In all cases left sterile and right infected. I. Moroccan phosphate; II. $CaHPO_4$

Fig. 1B. The same as Fig. 1A. III. $Ca_3(PO_4)_2$; IV. Bonemeal. (No. 18 probably infected during the experiment).

It is evident that in all these cases the plants in the infected pots grew better and had absorbed more phosphate than those in the sterile ones.

That the effect on ferrophosphate and Algerian phosphate is far less important than on the calcium phosphates is to be expected from the nature of these phosphates.

These results tend to point in the direction that microorganisms are able to make hitherto inaccessible phosphates available to the plant.

The question, however, arises as to what extent the better development of the plants also could be the result of the general activity of different groups of soil bacteria, with an increased phosphate intake as a logical consequence of the better growth and the expanded rootsystem.

Though soil microbes by some hitherto unknown activity might perhaps cause the plants to grow a little better and absorb some more phosphate, it seems quite impossible that they are able to increase the weight of the plants with 72-188% and the phosphate intake with 79-324% in a culture medium which contains all the necessary nutrients in a soluble form, the nitrogen as nitrates and only the phosphates in an insoluble or difficultly soluble form. Under these circumstances such increases can only be brought about by microbiological solubilization of the phosphates.

This experiment has to be considered as a preliminary one, because it was carried out in the autumn, with the result that the growth of the oat plants was rather poor. In order to get more reliable information a similar experiment was carried out on a somewhat larger scale in the following year.

Second experiment with oats in sterile and in infected quartz sand in Mitscherlich pots (from 23/3 to 16/6). Each pot contained 5 kg sand, 1 l water, 2,5 g KNO_3, 1 g $Ca(NO_3)_2$, 0,1 g $CaSO_4$, 0,1 g $MgSO_4$ and 1 cc of the mixture of minorelements previously mentioned.

The pots were sterilized, half of them already mixed with 1% of a fertile soil whereas the other half were infected after sterilization.

The experiments were for the greater part made in triplicate, a few, however, in duplicate. After two months another 1,5 g of $Ca(NO_3)_2$ was added.

The results are summarized in table III.

This experiment has been complicated by manganese deficiency, especially in the infected cultures with no or difficultly available phosphates, some of which lagged behind the sterile ones. It has been proved by the author [17]) that the roots of plants, deficient in manganese, are easily attacked by different soil-microbes, which cause severe root rot and make the situation for the plants much worse than in the sterile cultures with the same inadequate amount of manganese.

This and the absence or inavailability of the phosphate are responsible for the fact that in the case of the blank, and the Moroccan and ferro-phosphates, the infected plants yielded less than the sterile ones.

In the other pots, however, where the phosphates were more available and the root system could spread throughout the pots, the plants could absorb all the manganese present in the sand and remained healthy. Here we see the results of the former experiment confirmed, particularly in the case of tricalcium phosphate, where, compared with the sterile pots, the increase in phosphate assimilated by the plants in the infected cultures was seven times as high as the increase in yield. With bonemeal and dicalcium phosphate the increase in yield and the increase in assimilated phosphate are both of the same order of magnitude, so that we may assume that in these cases also the better growth was caused by an increased availibility of the phosphates.

The behaviour of the KH_2PO_4 series is interesting. It was expected that the intake of the phosphate from this soluble compound would be independent of the activity of the soil microbes so that a resulting increase in yield would indicate that, besides their solubilizing activity, the microorganisms exerted some other beneficial influence on the plants. We see that there is but a small increase in yield of only 3,8 % which lies within the probable error. Hence we may conclude that outside the phosphate range, there is no bacteriological activity which favourably influences the growth of the oat plants in our cultures.

Nevertheless the infection increased the phosphate intake by 27,3 % notwithstanding the fact that the phosphate was given as KH_2PO_4. It is to be noted that nitrogen was added as $Ca(NO_3)_2$ and KNO_3; under these circumstances KH_2PO_4 and $Ca(NO_3)_2$ will

TABLE III

Yield and phosphate content of oat plants in sterile and infected quartz sand (23/3–10/6)

Phosphate source	Dry weight of plants in g p. pot.	Mean	Increase in yield by infection in % of sterile	mg P_2O_5 assimilated by 5 plants	Mean	Increase in P_2O_5 assimilated, after infection in % of sterile	pH	Remarks
Blank, sterile	8.7			18.9			7.25	
	10.6	9.6		22.9	21.0		7.40	
	9.5			21.3			7.38	
Blank, infected *)	7.7			17.1			7.05	
	6.7	7.1	— 26 1	16.2	15.7	— 25.2	7.10	Manganese
	6.8			13.9			7.22	deficiency
Moroccan, sterile	8.7			21.6			7.25	
	10.5	9.6		22.7	22.1		7.55	
Moroccan,	8.6			20.4			7.45	
infected *)	8.2	8.4	— 12.5	23.5	22.0	0.0	7.38	Manganese deficiency
KH_2PO_4, sterile	20.5			248.7			7.10	
	19.2	20.6		226.2	232.6		7.45	
	22.0			222.9			7.00	
KH_2PO_4, infected	18.9			290.3			7.30	
	21.3	21.4	+ 3.8	288.2	296.1	+ 27.3	7.28	
	24.1			309.2				
Bonemeal, sterile	17.0			152.7			7.40	
	17.5	16.5		144.4	143.5		7.28	
	15.1			133.3			7.38	
Bonemeal,	22.1			215.9			7.33	
infected	24.6	25.4	+ 54.0	232.0	235.4	+ 63.0	7.45	
	27.5			258.2			7.43	
$Ca_3P_2O_8$, sterile	23.7			179.4			7.48	
	18.4	21.1		168.2	173.8		7.33	
$Ca_3P_2O_8$, infected	23.8			283.0			7.25	
	22.3	23.1	+ 9.5	296.5	290.0	+ 67.0	7.19	
$CaHPO_4$, sterile	18.4			224.7			7.25	
	19.5	17.5		266.7	225.7		7.33	
	14.5			185.7			7.33	
$CaHPO_4$, infected	25.2			410.5			7.25	
	25.0	25.5	+ 4.57	297 5	336.9	+ 49.7	7.43	
	26.3			302.7			7.28	
Ferro-phosphate	13.5			47.5			7.43	
sterile	12.5	12.7		32.6	40.1		7.30	
Ferro-phosphate,	10.5			34.9			7.40	
infected *)	10.7	10.6	— 16.5	33.1	34.0	— 15.2	7.48	Manganese deficiency

*) The plants in the infected pots showed typical symptions of manganese deficiency. By administering $MnSO_4$ the symptoms partly disappeared.

react to form $CaHPO_4$. As the plants grew, and NO_3' was replaced by CO_3'' the resulting $CaCO_3$ and K_2HCO_3 shifted the reaction still more in the alkaline direction, promoting the transformation of KH_2PO_4 into $CaHPO_4$.

However, $CaHPO_4$ is only slightly soluble (2 : 10.000); according to van den Honert[18]) the plant only absorbs phosphorus as H_2PO_4 ions, HPO_4 ions being inaccessible.

As the pH of the culture sand was about 7,3–7,2, it is to be expected that the equilibrium $H_2PO_4' \rightleftarrows HPO_4'' + H^+$ is shifted to the right, the concentration of the H^+ being very low at that pH.

At the other hand, especially because the concentration of HPO_4'' is low as well, it is evident that CO_2 or organic acids produced either by the roots or by microbiological activity in the rhizosphere, will soon produce enough H-ions to force the equilibrium back to H_2PO_4', which locally makes the phosphorus accessible to the plants. This experiment with oats strongly supports the supposition that microorganisms play a much more important rôle in these processes than hitherto was accepted. In order to obtain more evidence the same experiment was repeated with some other plant species.

Third experiment, with mustard (*Sinapis alba*) in sterile and infected quartz sand (from 8/5 to 28/6). The outline of this experiment was the same as that of the previous one; in each pot three mustardseeds were planted, all the cultures being made in triplicate. As phosphates, tri- and di-calcium phosphate, bonemeal, Moroccan phosphate and a mixture of KH_2PO_4 and Na_2HPO_4 with a pH of 6,0, were used.

The results are summarized in the following table.

In this series we noticed that in most of the infected pots the plants already showed after three weeks a pronounced chlorosis and remained small, whereas those in the sterile pots were green and were of a more robust appearance. The difference was most pronounced in the pots with $CaPHO_4$. This was reflected in the rate of evaporation, which in the sterile pots was three times as large as in the infected ones (123 cc as against 43 cc in 24 hours) (Fig. 2).

As the symptoms were those of a typical iron chlorosis, the plants were given an extra dose of ferricitrate (25 mg p. pot) with which to some extent they recovered, but not sufficiently to use this

TABLE IV

Yield and phosphate content of mustard, grown in sterile and infected quartz and, with different phosphates *) (8/5–28/6)

	Dry weight of plants in in g p. pot	Increase in yield by infection in % of sterile	mg P_2O_5 assimilated by the 3 plants	Increase in P_2O_5 absorbed by the plants by infection in % of sterile
Blank, sterile	0.7			
Blank, infected	0.3			
Bonemeal, sterile	12.8	— 14.1	116	— 12.9
ditto infected	11.0		101	
$Ca_3(PO_4)_2$, sterile	11.7	+ 13.9	116	+ 12.9
ditto infected	13.6		131	
$CaHPO_4$, sterile	10.4	— 65.5	143	— 60.9
ditto infected	3.6		56	
KH_2PO_4, sterile	10.9	— 9.9	137	— 13.1
ditto infected	9.8		119	
Moroccan, sterile	6.2	— 16.2	39	+ 8.8
ditto infected	7.2		42	

*) All these yields and determinations are the mean of 3 different cultures.

Fig. 2. Mustard (*Sinapis alba*) in quartz sand with $CaHPO_4$. The infected plants (No. 12) remain small because of iron deficiency, caused by precipitation of ferriphosphate in the rhizosphere. No. 11 Sterile plants.

series as a reliable criterium for the influence of microorganisms on the intake of insoluble phosphates by *Sinapis alba*. However, it is to be noted that the plants, which had received $Ca_3(PO_4)_2$ and Moroccan phosphate absorbed more phosphate (12.9 and 8.8% resp.) notwithstanding the dryweight in both cases had decreased with 13,9 and 16,2% resp.

In order to obtain more evidence to show that this trouble was caused by an iron deficiency, in some way connected with bacteriological activity, the following experiment was carried out.

Increasing quantities of iron (a mixture of ferricitrate and ferrichloride) were added to two series of glassculture vessels containing $1\frac{1}{2}$ kg of sand, sterile and infected. The following nutrients were added to each pot: 0,3 g KNO_3; 0,3 g $Ca(NO_3)_2$; 0,6 g $CaHPO_4$; 30 mg $CaSO_4$; 60 mg $MgSO_4$; 0,3 cc of the stock mixture of minor elements, 300 cc H_2O and 1,6, 4, 8, 16, and 32 mg Fe''' respectively.

After one month the plants showed marked differences and were photographed. (Fig. 3).

When we compare the plants in the infected pots containing the smallest amount of iron (No. 1 with 1,6 mg and No. 2 with 4 mg)

Fig. 3. Sterile (Nos. 6–9) and infected (Nos. 1–5) cultures of mustard in quartz sand $Ca_3(PO_4)_2$, with increasing quantities of iron. (resp. 1,6 mg, nos. 1 and 6; 4 mg, nos. 2 and 7; 8 mg, nos. 3 and 8; 16 mg, nos. 4 and 9; 32 mg, no. 5. Iron as a mixture of ferricitrate and ferrichloride. Comparing no. 1 with no. 6 the sterile plant is much larger than the infected one, the same as in fig. 2. By increasing the quantity of iron, the situation is reversed, and the infected plants become larger than the sterile ones. (See text).

with the sterile ones containing the same amounts (No. 6 and No. 7), we see that in both cases the sterile ones were much larger. The plants in the infected pots with the greater amounts of iron however, (Nos. 3 with 8 mg and 4 with 16 mg) stood better and were larger than those in the sterile ones. (No. 5 excepted, where the iron-content proved to be too high for optimal growth). It seems possible that these remarkable differences are connected with the iron-phosphate equilibrum in the sand culture. In the infected pots phosphate is brought into solution by the microorganisms in the rhizosphere; when only small amounts of iron are present, the greater part of it will be precipitated as ferriphosphate which is insoluble and a very poor source of iron for the plant. It is to be expected therefore that under these circumstances the plants in the infected cultures suffer from iron chlorosis.

In the sterile cultures with the same small amount of iron there is no microbiological solubilization of phosphates and consequently no precipitation of the iron in the rhizosphere. Here the greater part of the iron is available to the plant. When, however, larger amounts of iron are added to the sterile cultures (Nos. 7, 8, 9) containing difficultly soluble phosphate this may give rise to another complication, namely the precipitation of the available phosphate as ferriphosphate, which is a very poor source of phosphate. As no phosphate is made available by microbiological activity, the plants in the sterile pots will suffer from phosphate deficiency, when increasing amounts of iron are added.

To what extent the plants will suffer either from iron deficiency or phosphate deficiency or from both depends on the final equilibrum between the different ions as influenced by their concentration, the solubility of the compounds used, microbiological activity in the rhizosphere and the pH. *)

Fourth and fifth experiment in sterile and infected quartz sand (with Mustard, *Sinapis alba*, and

*) An anologous iron-phosphate antagonism has been observed by the author in reduced soils; by microbiological reduction the insoluble ferric compounds are transformed into soluble ferro-hydrocarbonate which reacts with the phosphates in the soil solution iorming ferrophosphate which to some extent is still available to the plants. When the soils are oxidized afterwards, the ferrous phosphate is transformed into ferriphosphate and the plants then suffer from a phosphate deficiency.

Sunflower, *Helianthus annuus*, as test plants). In order to minimize the above-mentioned complications the concentration of the iron in the following experiment was increased to 100 mg ferric citrate per pot, whilst $Ca_3(PO_4)_2$ was used as the source of phosphate in order to keep down the concentration of the HPO_4-ion as low as possible. Under these circumstances it was expected that sufficient iron would be available, whilst the plants would readily respond to any increase in available phosphate, subsequently caused by microbiological activity.

To 5,5 kg of quartz sand the following nutrients were added: 1,8 g KNO_3; 1,2 g $Ca(NO_3)_2$; 0,1 g $CaCO_3$; 0,1 g $CaSO_4$; 0,2 g K_2SO_4; 0,5 g $Mg(NO_3)_2$. Minor elements: 50 mg $MnSO_4$; 2 mg $CuSO_4$; 2 mg H_3BO_4; 2 mg $ZnSO_4$; 0,5 mg KI; 0,1 mg Amm. molybdate, and 100 mg ferricitrate. Three sterile mustard-seeds per pot. In order to prevent infection of the sterile cultures when watering the pots, a device was set up which enabled us to

TABLE V

The influence of infection on the phosphate intake and growth of mustard plants $(Ca_3)(PO_4)_2$ (1/8–17/10)

	Length of the plants p. pot in cm (average of 3 plants)	Dry weight of the plants per pot (g)	mg P_2O_5 assimilated by 3 plants per pot	pH
Sterile I	28.5	10.0	65.0	6.8
II	70.5	11.7	111.1	6.9
III	27.6	15.1	111.7	6.9
IV	34.5	8.5	63.8	6.8
Mean	**40.3**	**11.3** ± 1.4 *)	**87.9** ± 13.6	**6.8**
Infected I	90.9	18.0	119.6	6.7
II	111.0	22.5	159.8	6.9
III	100.5	14.0	106.4	6.8
IV	90.8	19.7	143.8	6.8
Mean	**98.4**	**18.6** ± 1.8	**134.9** ± 12.0	**6.8**
Mean increase by infection in % of the sterile ones	+ **144%**	+ **64.6%**	+ **53.6%**	

*) The mean difference between infected and sterile is 7.3 g for dry weight and 47 mg P_2O_5. The odds in favour of these differences are sufficiently significant to regard them as real.

Fig. 4. Mustard in quartz sand ($Ca_3(PO_4)_2$). In this case special care has been taken to ensure an adequate iron supply. The infected plants (upper) are larger than the sterile ones (lower), indicating that phosphate has been made available by the bacteria in the rhizosphere.

keep the water content of the sand automatically constant without any supervision during the time of the experiment. The principe of this ingenious and simple glassapparatus for the continuous watering of pot cultures was developed by Calfee and McHargue[18]) and Offset[19]); except for a few improvements which will be reported elsewhere, we used the same set-up.

The sterile plants very soon lagged behind the infected ones which bloomed earlier and subsequently grew much higher, as can be seen in the photograph.

In this experiment we see a distinct increase in length, weight, and phosphate intake of the plants as a result of the infection. In

Fig. 5. Sunflower plants (*Helianthus annuus*) in quartz sand ($Ca_3(PO_4)_2$). The infected plants (right) bloom 2–3 weeks earlier than the sterile ones. (left).

order to check these observations the experiment was repeated, this time including sunflower as a second test plant.

Again in this experiment the infected mustard plants bloomed 3-4 weeks earlier than the sterile ones, and the infected *Helianthus* plants were considerably larger than the sterile ones. (See photograph No. 4 and 5).

In both cases the yields were more than doubled by the infection, whilst at the same time there was a considerable increase in the quantities of P_2O_5 absorbed by the plants.

However, it must be noted that in this experiment as well as in the previous ones, notwithstanding the quantities of phosphate absorbed by the plants increased considerably by infection, the *sterile* plants also absorbed notable amounts of phosphorus from $Ca_3(PO_4)_2$ and bonemeal. The solubility of these substances being negligible, one must assume that the roots of oats, mustard- and sunflower plants are able to mobilize part of the phosphorus of these substances and make them to some extent available, independant of microbiological activity. As none of the previous investigations on this subject has been carried out under sterile conditions, the definite proof for this wide spread assumption was still lacking.

Some experiments with basic-slag in quartz sand and virgin heath-soil (sterile as against

TABLE VI

Yield and phosphate content of mustard and sunflower plants grown in sterile and infected quartz sand $Ca_3(PO_4)_2$ (20/4–19/6)

Description	Length of the plants per pot average in cm	Dry weight per pot (g)	Increase in yield by infection in % of sterile	Total P_2O_5 assimilated per pot mg	Increase in P_2O_5 assimilated by infection in % of sterile	pH
Mustard, sterile I	98	12.5		97.5		6.6
,, ,, II	72	9.0		70.3		6.8
the same, infected I	111	22.0		154.0		7.0
,, ,, ,, II	134	23.0	108.3%	171.0	93.9%	6.8
Helianthus, sterile I	61	12.0		50.4		6.6
,, ,, II	65	22.5		83.2		6.5
the same, infected I	97	35.0		119.0		6.7
,, ,, ,, II	105	41.5	121.5%	124.0	82.0%	6.5

infected). In general outline these experiments were the same as the previous ones, except that phosphate was administred as basic-slag (5 g per pot).

The experiment in quartz sand, both sterile and infected with 1% soil, lasted from 27/5–17/8; the second experiment with virgin heath soil lasted from 12/8–17/11. In the latter case a comparison was also made with an equivalent amount of superphosphate not infected. All experiments were made in triplicate and all pots were sown with 5 oat seeds.

The results are summarized in table VII.

Both experiments demonstrate a marked increase in phosphate intake and dry weight of the plants, caused by infection; the enormous increase in yield and phosphate absorbed as a result of the use of superphosphate clearly demonstrates that this soil is highly deficient in phosphate and it is evident that in the infected pots the increase in weight and the increased phosphate intake are caused by the solubilizing activity of the added soil microbes on the basic slag. As the original heath soil in this experiment was not

TABLE VII

Experiment with basic slag in quartz sand and heath soil (oats)

		Dry weight g per pot	Mean	Increase in yield in % of sterile or original	mg P_2O_5 assimilated by 5 plants	Mean	Increase in P_2O_5 assimilated in % of sterile or original	pH
Quartz sand, sterile	I	6.0			28.6			7.3
	II	8.7	6.5		61.2	39.2		7.2
	III	4.8			27.7			7.2
Quartz sand, infected	I	10.4			60.1			7.3
	II	7.5	9.6	+ 47.7	44.2	57.3	+ 46.2	7.4
	III	11.0			66.6			7.3
Sandy heath-soil from Marum. Original soil	I	3.5			19.2			5.3
	II	3.8	3.2		19.4	17.5		5.2
	III	2.4			13.9			5.2
Same, infected with 1% fertile soil	I	5.9			29.5			5.6
	II	4.5	5.5	+ 72.0	21.6	26.1	+ 49.2	5.7
	III	6.2			27.2			5.6
Original soil, with superphosphate	I	10.5			64.0			4.8
	II	10.0	10.3	+ 222	67.0	65.8	+ 275	4.8
	III	10.5			74.5			4.8

sterilized, the experiment shows, that in this virgin soil, the number of phosphate dissolving microbes is apparently too low to provide for the requirements of the plants.

Although no general conclusions can be drawn from a couple of experiments, it is tempting to connect this result with the fact that on these virgin, sandy heath soils, basic slag has generally proved to be far inferior to superphosphate as a source of phosphate, notwithstanding the fact that these soils are acid. After a few years, however, the situation improves and basic slag can be used with fairly good results *).

The most commonly accepted formula for the phosphatic compound in basic slag is a double silicate and phosphate of lime $(CaO)_5P_2O_5SiO_2$; according to Lyon and Buckman [20]) this compound is quickly rendered available in the soil by carbon dioxide for which they suggest the following reaction:

$$(CaO)_5P_2O_5SiO_2 - 8CO_2 - 6H_2O = CaH_4(PO_4)_2 + 4CaH_2(CO_3)_2 + SiO_2.$$

This being the case it is to be expected that the micro organisms in the soil and especially in the rhizosphere, play an important rôle in making the phosphorus in basic slag available to the plant. Our experiments tend to point in the same direction.

Infection experiments with pure cultures of different soil bacteria. From the different pot cultures a number of bacteria and a few fungi were isolated by laying the roots of these plants together with the adhering sand particles on nutrient-agar.

The nutrient agar consisted of a decoction of carrots, (1 kg carrots per 1 tapwater) 0,1% asparagine, 0,1% glucose, 2% agar. After sterilizing, the agar was cooled to 50° C and quickly mixed first with 0,5 cc 10% K_2HPO_4 then with 1 cc 10% $CaCl_2$, a homogenous precipitate of $CaHPO_4$ being formed.

In order to prevent spreading of the bacteria over the surface of the agar, the plates were dried in the usual manner, and then incubated at 18° C.

After 2–3 days colonies developed around the roots, in some cases dissolving the phosphate; in other cases the opposite occurred

*) It must be emphasized that microorganisms, which solubilize phosphates, generally will be present in all normal soils; consequently inoculation, with the object to improve the phosphate intake by the plants, will be of no use.

and the precipitate became denser, as is shown by the accompanying photographs No. 6 A und B. From the raw cultures a number of pure cultures were isolated. The behaviour of these cultures could be classified as follows:

1. The phosphate is solubilized and remains so.
2. The phosphate is solubilized and is afterwards precipitated.
3. $CaHPO_4$ is transformed into $Ca_3(PO_4)_2$, the precipitate becomes denser and remains so.
4. As 3, but after some time the precipitate is dissolved.

The latter is characteristic of some of the isolated fluorescent bacteria, which were prevalent in a number of cases.

The somewhat contradictory behaviour of the above-mentioned bacteria is undoubtedly due to the fact that they are able to produce acid from the sugar in the nutrient medium as well as ammonia from asparagine and other nitrogen containing substances.

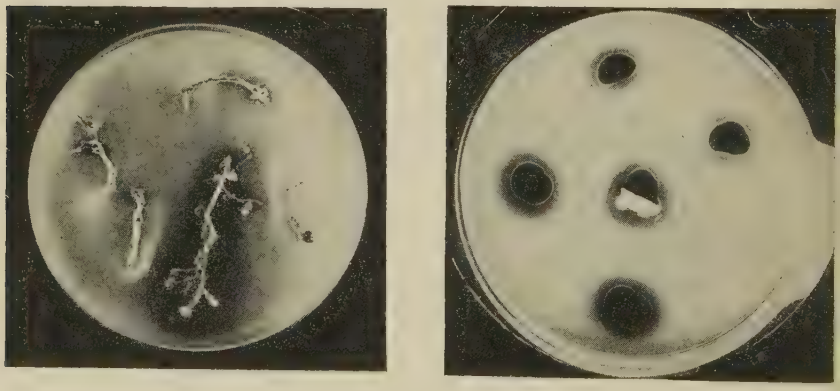


Fig. 6. The isolation of phosphate solubilizing bacteria. *A*. The roots from infected cultures are placed on nutrient agar, in which $CaHPO_4$ had been precipitated. In some cases the phosphate is solubilized, in other cases it is precipitated. Photographed with incident light. *B*. A bacterium which produces a dark blue colour, precipitates phosphate around the colony and dissolves it just beneath the colony. Half of the colony in the centre has been removed. Photographed with transmitted light.

Also interesting was the behaviour of a bacterium which produced large dark blue colonies and which precipitated phosphate around the colony, whereas immediately beneath the colony the phosphate had been dissolved, most probably by the carbon dioxide exhaled by the bacteria.

The equilibrum between dissolved calcium phosphate and precipitated calcium phosphate is governed by the pH; under the influence of microbial metabolism solubilization as well as precipitation may occur, depending on whether acid or alkali is produced. The way in which such bacteria behave in the rhizosphere depends largely on the substances excreted by the roots.

Since the number of different bacteria which could be isolated from the roots was so large, they have not been classified. Only a few active colonies were picked out and used in the following experiments. After being grown on nutrient agar, the colonies were suspended in tapwater and the suspension mixed with the sterile

TABLE VIII

Infection experiments with pure cultures of different microbes (oats in sterile quartz sand)

First experiment $^1/_6$–$^1/_9$ Description	Dry weight per pot g	Mean	Increase by infection in % of sterile	mg P_2O_5 assimilated per pot	Mean	Increase in P_2O_5 assimilated after infection in % of sterile
Sterile	21.8			298		
	21.6	21.1		307	304	
	19.8			307		
A. Infected with a fungus	25.7			350		
	24.6	25.2	+ 19.4	372	361	+ 18.8
B. Infected with phosphate dissolving bacteria	23.5			353		
	22.7	23.1	+ 9.5	355	354	+ 16.4
C. Infected with blue bacteria	17.7	17.7	— 16.1	327		
	—	—		285	306	+ 0.7
D. Infected with fluorescent bacteria	19.2			353		
	19.6	19.4	— 3.3	365	359	+ 18.4
Second Experiment. $^4/_8$–$^{30}/_{10}$						
Sterile	9.5			228		
	10.2	10.1		210	219	
	10.5			219		
E. Infected with another fungus	12.5			292		
	11.5	12.0	+ 19.7	242	267	+ 21.9
F. Infected with phosphate dissolving bacteria	12.2			297		
	14.0	13.1	+ 30.7	347	322	+ 54.0
G. Ditto	14.0			284		
	13.0	13.5	+ 34.8	306	295	+ 34.7

quartz sand which contained the same nutrients as in the previous experiments, phosphate being added as $CaHPO_4$, 2,1 g per 5 kg sand; 5 sterile oat seeds per pot.

Two successive experiments were carried out, the first from 1/6–9/9 and the second from 4/8–30/10 (table VIII).

In 5 out of 7 cases we see a notable increase in weight of the plants, whilst in 6 cases the phosphate intake by the plants has been favourably influenced by infection.

Particularly convincing is case F, where the increase in phosphate absorbed by the plants is 1,75 times the increase in weight of the plants and case D, where the phosphate intake increased with 18,4% and the weight of the plants practically remained unchanged.

These experiments with pure cultures confirm the results obtained with crude infections, so that we may assume that microorganisms in general play an important rôle in the phosphate intake of different plants from insoluble or difficultly soluble phosphates.

pH changes as influenced by infection. As the solubilization of the phosphates largely depends upon the lowering of the pH in the rhizosphere and perhaps in the surrounding soil as well, the pH has been determined in a number of cases.

At the conclusion of the experiments the sand, adhering to the roots, was suspended in distilled water and the pH then determined.

Also a sharp Pt needle electrode was used covered with quinhydrone, to determine the pH in the rhizosphere. To ensure a reliable contact for the calomel electrode a glass tube of 1 cm diameter was pushed into the wet sand contained in the Mitscherlich pots and some molten, saturated KCl agar was then poured into the tube on top of the sand. After solidification some KCl solution was poured onto the agar, and in this was placed the calomel electrode.

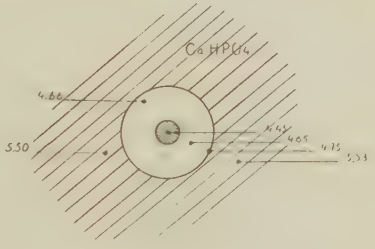
It is evident that the pH changes in the sand or soil surrounding the roots are of little importance. In a number of other cases the pH in the rhizosphere, as determined with the needle electrode, was compared with the pH in the same culture vessel at some distance from the roots. Only in the case of bonemeal was the pH in the rhizosphere 0,5 pH lower than that of the rest of the sand; in the case of $Ca_3(PO_4)_2$, however, just the reverse was found.

TABLE IX

Average pH's in different cultures, sterile compared with infected, at the conclusion of the experiments

Description	Sterile	pH infected	Difference
Second exp. with oats Blank	7,34	7,13	— 0,21
Moroccan phosphate	7,40	7,42	0,02
KH_2PO_4	7,18	7,29	0,11
Bonemeal	7,35	7,40	0,05
$Ca_3(PO_4)_2$	7,40	7,22	— 0,18
$CaHPO_4$	7,30	7,32	0,02
Ferro-phosphate	7,37	7,44	0,07
4th experiment, with mustard $Ca_3(PO_4)_2$	6.95	6,80	— 0,15
5th exp. ditto	6,71	6.90	0,21
Experiment with sunflowers	6,7	6,60	0,10
Exp. with oats in quartz sand (basic slag)	7,23	7,33	0,10
Ditto in sandy heath soil	5,23	5,63	0,40

In order to obtain an impression of the pH range around a colony of phosphate dissolving bacteria on nutrient agar in which $CaHPO_4$ has been precipitated, the pH was determined with the needle electrode at different places inside and outside the clear zône.



The pH's are relatively low, but it is evident that the solubilization can be brought about by carbon dioxide, which has a pH of 3,8 in a saturated solution at room temperature.

Biological CO_2 production, however, has to be rather intensive for the production of such low pH's.

That the results of the pH measurements were of little importance is after all not so startling, especially in the light of the experiments mentioned in the following chapter, which showed that solubilization in the rhizosphere is often localized in a number of distinct spots along the roots.

Moreover the picture is confused since in most cases the plants grew much better in the infected pots than in the sterile ones. This causes an extra shift in the pH towards the alkaline side, especially when nitrogen has been added as KNO_3 and $Ca(NO_3)_2$, from which the basic part is left behind after the nitrogen has been absorbed.

Direct observation of phosphate solubilization by soil microbes. To complete our experiments it seemed advisable to try to obtain more direct proof of the solubilizing activity of the soil microorganisms in the rhizosphere.

Fig. 7. The device used for demonstrating the solubilization of the phosphates in the rhizosphere. A glass plate covered with an agar film, in which calciumphosphate had been precipitated is put aslant in a cylindrical culture vessel filled with quartz sand + nutrients, sterile or infected.

Sterile oval shaped glass plates were covered with a layer of 2% agar, containing 2% KH_2PO_4. With the necessary precautions to maintain sterility, the plates were bathed in a solution of 5% $CaCl_2$, which resulted in the formation of a uniform precipitate of $CaHPO_4$.

In order to prevent the agar film from slipping off the plates, small grooves were filed at the edges of the plate at a distance of 2–3 cm from each other. In these groves strong white yarn was laid, fitting tightly around the plates.

The oval plates fitted closely into the cylindrical culture vessels of 3 litre capacity; dry sterile quartz sand, mixed with nutrients was then poured onto the plates, the pots were watered through a glass tube, buried in the sand.

As in the first experiment with oats only a few solubilization spots could be found, no convincing proof of the solubilizing activity of the microorganisms could be obtained in this way.

However, the oatplants in the former experiments had shown a marked response to infection, so it may be possible that in this case the excretion of organic substances is scanty and consequently the activity of the microbes in the rhizosphere is strictly limited to a shallow zône.

As a second test-plant I chose Rape, (*Brassica Rapa* and *Brassica Rapa var. annua*), known for its rich growth and large foliage, which probably warranted a more abundant root excretion. With these plants the results were very convincing, as can be seen in the photographs (fig. 8 A. and B.). After six weeks the plates were covered with a thick felt of roots; when the majority of the roots had been cut away it could be seen that underneath numerous roots small but distinctly clear solubilization zónes had been formed. A few facts are particularly worthy of note: these clear zônes were scattered at random all over the plates; there were also roots, underneath which nothing could be seen whilst beneath an odd number of roots narrow white precipitation zónes could be detected *).

This somewhat whimsical behaviour is doubtless due to the fact that these phosphate dissolving microorganisms are not omni-

*) It is to be expected that during the microbiological deamination of organic substances with a low $C : N$ quotient, as aspargine and leucine, ammonia will be liberated, which temporarily causes the precipitation of the phosphate. Eventually, however, when these substances are completely decomposed, the quantity of CO_2 produced is sufficient to neutralize the ammonia.

Consequently microbiological precipitation of phosphates in the rhizosphere will be rather an exception.

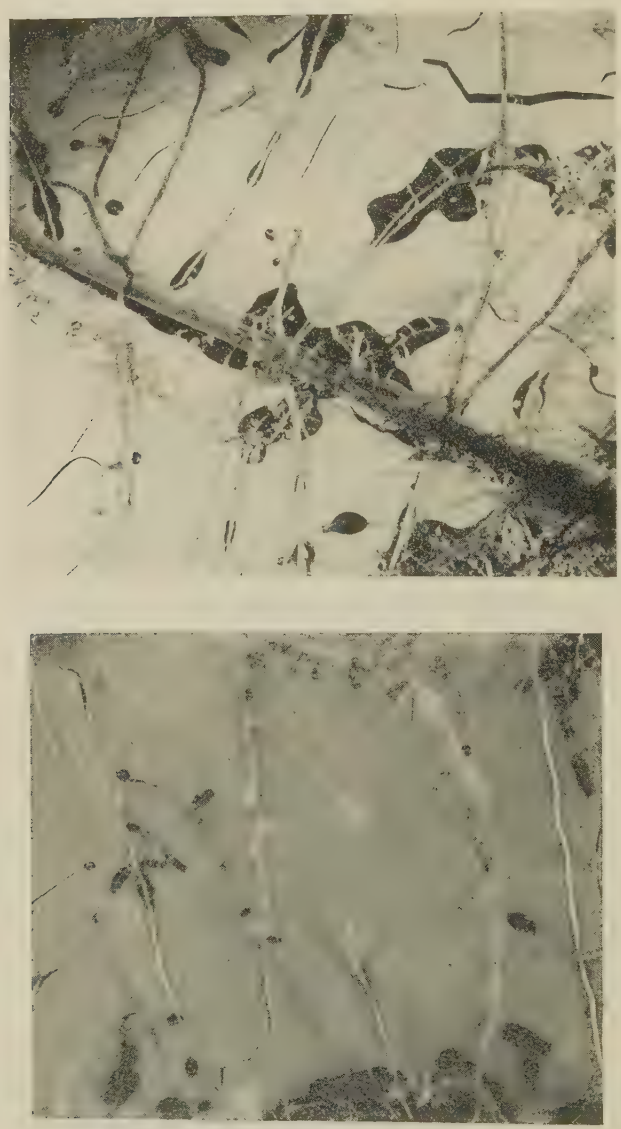


Fig. 8. *A* (upper) Microphotograph of the surface of the agar-glass plate; note the localized solubilization zônes at certain spots along the roots. (The rope in the middle is yarn, to prevent the agar from slipping of the plate). *B* (lower) In this microphotograph it is to be observed that besides a number of transparent solubilization spots, especially around the root tips and young branches, there are distinct white precipitation zônes along and beneath other roots.

present, but are located at certain spots on the roots, where they multiply and develop into colonies. The same holds true for the phosphate-precipitating bacteria.

These observations were made during the first experiment which lasted from 8 4 until 23 5, a rather cool period in our country.

After the second experiment, which ran from 12 6 until 1 8, it was found that, with the exception of some isolated spots, the phosphate in the plates was completely solubilized. It is evident that the increased activity of the microbes in this case is a natural consequence of the higher summer temperatures.

In the sterile pots the phosphate did not show any signs of solubilization and one is forced to suppose that in the case of *Brassica Rapa*, the rootexcretions as such, in the absence of bacteria, do not play a rôle of importance in the phosphate intake by the plants. Consequently the roots of the infected plants were much stronger developed than those of the sterile plants.

From these experiments the general conclusion can be drawn that microorganisms in the rhizosphere may play a very important rôle in making insoluble or difficultly soluble phosphates available to the plant.

To what extent this will happen depends on different factors, of which the following may be mentioned: the plant species, the nature and the quantity of the root excretions, the presence and number of phosphate-solubilizing microorganisms in the soil (low in newly reclaimed heath and peat soils), the character of the phosphates used, the pH and the temperature of the soil.

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Summary and conclusions

With a view to obtain definite information regarding the influence exerted by microorganisms on the phosphate intake by the plants, oats, mustard, sunflower and rape were grown under sterile and infected conditions in pot cultures.

Insoluble or difficultly soluble phosphates were added as Algerian phosphate, Moroccan-phosphate, bonemeal, tri- and dicalcium phosphate

and ferrophosphate. In order to eliminate the influence of nitrification, nitrogen was administered as nitrate.

The fact that in many cases the amount of phosphorus assimilated by the plants as well as the weight of these plants increased markedly by infection, proves that the microorganisms had definitely made available a certain amount of phosphorus, otherwise inaccessible.

That in a number of cases the infected plants had absorded much more phosphate than the sterile ones, whereas the increase in weight was negligible, proves that there was no favourable bacteriological activity besides the solubilizing of the phosphates.

In a number of sterile cultures, however, the amount of phosphorus assimilated by the plants from substances like $Ca_3(PO_4)_2$ and bonemeal, though much smaller than in the infected cultures, was quite considerable. This proves definitely that in the absence of microorganisms the roots may take an active part in solubilizing difficultly soluble phosphates.

In one case the weight of the infected mustard plants was much smaller than that of the sterile ones; this could be traced to iron chlorosis, caused by the precipitation as ferriphosphate of the rather small amount of iron in the culture solution, by the phosphate, solubilized by the bacteria in the rhizosphere. By using a culture solution containing more iron (as ferricitrate) and administering the phosphate as $Ca_3(PO_4)_2$ in stead of $CaHPO_4$, the infected mustard plants grew much better, bloomed three weeks earlier and absorbed more phosphate than the sterile ones.

Special attention is drawn towards the phosphate-iron equilibrium as influenced by microbiological processes in the rhizosphere as well as in the soil. (Reduction and solubilization of excessive amounts of iron in waterlogged soils, immobilizing the phosphate ions.)

Infecting sterile cultures of oats (P added as $CaHPO_4$) with pure cultures of bacteria, isolated from the roots of different plants, resulted in an increased phosphate intake varying from 16 to 54% and an increased dry weight of most of the plants.

Some evidence has been obtained that the intake of phosphorus from basic slag is markedly improved by the activity of soil microbes. This may be of importance in relation to the nature of the phosphates which have to be added to newly reclaimed, virgin heath and peat soils.

Direct proof of the solvent action of microorganisms in the rhizosphere on insoluble phosphates was obtained with the aid of glassplates, covered with an agar film in which calcium phosphate was precipitated and which were buried aslant in the culture vessels. Clear solubilization zônes were observed in distinct spots, especially underneath root tips and young branches of *Brassica Rapa* and *Brassica Rapa var. annua*.

In the sterile cultures these solubilization zônes were absent, proving that in this case the solvent action of the roots was negligible.

In rare cases, especially when the roots excrete organic substances with a low C : N quotient, there is a possibility that temporarely phosphates are precipitated by microbiological activity in the rhizosphere.

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REACTION CHANGES IN THE LIFE-MEDIUM OF PLANTS CAUSED BY DIFFERENTIAL INTAKE OF IONS

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The nature of so called physiollogically acid or alkaline fertilizers is a well known fact. The preferential intake of the cation in ammonium salts [1], [2] and of the anion in the case of nitrates [1–4] and the resulting influence on the reaction of the life-medium has been studied by various authors. Investigations into the intake of sodium chloride and potassium chloride, however, have hitherto mainly shown a practically equal intake of anion and cation [1–7]. This has in our view to be attributed to the fact that of these salts low concentrations only were employed. Our investigation shows a preferential anion-intake with these salts resulting in alkalinisation of the medium. This observation, being a continuation of earlier work carried out in our laboratory [8], [9] seems to be of particular interest for irrigated agriculture in arid countries and whereever a strong accumulation of chlorides occurs in the soil.

Contrary to the usual methods used in investigations in this field, i.e. raising the plant first in a complete nutrient solution [10–17], or in a solution containing a mixture of salts [18], barley was grown from the germination of the seed onwards in single-salt solutions. It was thus found possible to eliminate mutual interaction of salts and to trace the influence of a single salt in each particular case. We succeeded in growing the seedlings for a period of 18 days and although symptoms of malnutrition were observed, no extreme ill-effects resulted and consistent results were obtained.

The present paper describes:

1. The development of barley plants grown in single-salt solutions using KCl, NaCl, NH_4Cl, $Ca(NO_3)_2$ and K_2SO_4.

2. The different behaviour of cation and anion-intake and exchange according to the nature of the salt solution and its concentration.

3. Changes in the hydrogen-ion concentration of the salt solution caused by preferential cation-or anion-intake.

4. The role of the respiration process in the preservation of an electrostatic equilibrium with special reference to concentrated solutions of NaCl, KCl and $Ca(NO_3)_2$.

Experimental Part. The solutions in which the barley seedlings were grown contained the monovalent salts NaCl, KCl, NH_4Cl or the divalent salts $Ca(NO_3)_2$ and K_2SO_4. In the case of each salt the experiment was carried out in four different concentrations, namely, 0.1*n*, 0.05*n*, 0.01*n* and 0.005*n*. The seedlings grew in these solutions for a period of 18 days. Changes in the pH of the solutions were constantly recorded. At the end of the experimental period the quantity of bicarbonates was determined volumetrically. The plants were then dried and analysed. In the following table the composition of the ash of the barley seeds used in this experiment is given:

TABLE I

Composition of the ash of barley-seeds (M.E. 10^{-1} in 1 gr)							
Na	K	Ca	Mg	Cl	HPO_4	SO_4	N
0.39	1.11	0.45	1.10	0.21	2.31	0.30	9.71

The quantity of ions absorbed from the salt-solutions was calculated by subtracting the quantity found in the seeds from that determined in the ash of the seedlings (100 seedlings in each case) after the period of growth. All analytical results are given in milli-equivalents $.10^{-1}$ per gram dry matter of the plant.

For the determination of Na and K the plants were ignited after the addition of concentrated sulphuric acid. Sodium was precipitated by zinc uranyl acetate according to Barber and Kolthoff[19-21]). Potassium was determined in the presence of sodium cobalt hexanitrite according to the modification of Katakousinos–Papadimitriou[22]). For the determination of phosphoric acid and sulphuric acid plants were ignited in the presence of $Ca(OH)_2$ and calcium acetate. Phosphoric acid was determined by the colorimetric method of Zinzadze[23]) and sulphuric acid by the usual gravimetric method. Calcium was determined by the method of Kramer–Tisdall[24]) and magnesium by

Hibbard's [25]) volumetric method. Nitrogen was determined by the Kjeldahl method, as modified by Jodlbaur and chlorine was titrated according to Volhard after wet oxidation of the plant material.

All experiments were carried out at least in duplicate and in each experiment the analytical determinations were repeated as well. All values given in the tables are the averages thus obtained.

1. *Weight and development of barley seedlings grown in different singlesalt solutions of various concentrations.* Table II shows the influence of different salt solutions upon the development and weight of seedlings at the end of the growing-period. (calculated to 100 seedlings). It will be observed that for each concentration the highest fresh-weight is found for plants grown in KCl solutions. If we compare, on the other hand, the fresh weights of the seedlings which

TABLE II

The differences in the development of seedlings growing in various salt solutions of various concentrations

Solution	Concentration of the solution	Fresh weight in g	Dry weight in g	Length of leaves in cm	Length of roots in cm	Number of seedlings
KCl	0.1*n*	22.0	2.72	11.0	4.0	94
	0.05*n*	29.5	2.68	17.2	5.8	100
	0.01*n*	26.5	2.44	18.6	4.7	97
	0.005*n*	26.6	2.39	17.9	4.2	100
NaCl	0.1*n*	17.6	3.74	5.6	2.3	88
	0.05*n*	20.8	3.12	9.5	3.2	93
	0.01*n*	25.8	2.75	13.1	3.5	91
	0.005*n*	22.0	2.76	13.4	3.3	97
NH_4Cl	0.1*n*	16.0	2.33	5.3	2.5	94
	0.05*n*	17.9	3.10	8.3	2.0	84
	0.01*n*	20.0	2.49	11.2	2.0	80
	0.005*n*	19.1	2.54	11.0	1.5	85
K_2SO_4	0.1*n*	19.9	2.66	11.6	2.0	99
	0.05*n*	20.6	2.74	11.5	2.2	97
	0.01*n*	18.9	2.72	11.9	2.2	99
	0.005*n*	17.9	2.65	12.0	2.3	96
$Ca(NO_3)_2$	0.1*n*	15.9	2.18	9.0	2.4	94
	0.05*n*	19.3	2.53	12.3	3.7	99
	0.01*n*	19.7	2.60	12.7	3.2	96
	0.005*n*	19.9	2.38	11.5	3.2	95

developed in solutions of the two potassium salts, i.e. KCl and K_2SO_4 we see that the values in the former series greatly surpass those found in the solutions of K_2SO_4. In relation to the dry weight of the seedlings, no great influence is shown, either by the different salts or by the various concentrations. With regard to the number of plants which developed from the 100 seeds sown in each experiment we see that in the majority of cases between 94 and 100 developed. The best crop was obtained from solutions of KCl and K_2SO_4 and the poorest from NH_4Cl solution.

Concerning the influence of different salts upon the length of the barley leaves in these experiments, there appears here the same gradation as for the fresh weight of the whole crop. Nevertheless, the influence of the concentration of the solution on the length of leaf is here more sharply emphasized. Relatively long leaves developed in dilute solutions (without appreciable differences between concentrations of 0.005*n* and 0.01*n*), and their length continually decreases with an increase in the concentration of the solution. An exception is formed by the length of leaf of seedlings grown in a solution of K_2SO_4. Here no influence of the concentration of the solution on the length of the leaf is seen.

With regard to the average length of roots of the seedlings, too, the gradation follows the same general line as in the case of the length of the leaf.

2. *The process of ion absorption by seedlings in various single-salt solutions.* A perusal of Table III enables us to arrive at the following conclusions:

The plant absorbed equivalent quantities of cation and anion only from dilute solutions, i.e. 0.01*n* and 0.005*n*, of NaCl and KCl. In all other cases there was an unequal absorption of varying character.

In all the experiments with NH_4Cl and K_2SO_4 the absorption was dominantly cationtic. In the case of $Ca(NO_3)_2$ the absorption was dominantly aniontic at all concentrations. A particular sensitivity of the plant regarding the character of the absorption was revealed in the experimental series with NaCl and KCl.

As already mentioned before, the absorption from dilute solutions of NaCl and KCl was equivalent, but at higher concentration, i.e. from 0.05*n* onwards, the character of the absorption changes into a one-sided aniontic one. The finding of a turning-point in the ab-

TABLE III

Solution	Concentration	Ion absorption by Barley-seedlings from various salt solutions (10^{-1} M.E. per gram dry matter): Cations absorbed	Anions absorbed	Difference between the two ions	Total of the two ions	pH values of the solutions at the beginning and at the end of the experiment: pH Beginning	pH End *)
KCl	0.1*n*	17.0	22.3	— 5.3	39.3	6.2	8.2
	0.05*n*	14.6	19.4	— 4.8	34.0	6.2	8.1
	0.01	11.5	11.0	+ 0.5	22.5	6.1	4.5
	0.005*n*	7.5	7.4	+ 0.1	14.9	6.6	4.4
NaCl	0.1*n*	10.6	12.8	— 2.2	23.4	6.9	8.9
	0.5*n*	10.0	10.6	— 0.6	20.6	6.7	8.7
	0.01*n*	7.3	6.2	+ 1.1	13.5	6.6	4.3
	0.005*n*	4.1	3.2	+ 0.9	7.3	6.9	4.5
NH_4Cl	0.1*n*	10.9	6.4	+ 4.5	17.3	5.5	4.4
	0.5*n*	18.1	6.7	+ 11.4	24.8	5.4	4.6
	0.01*n*	4.3	1.3	+ 3.0	5.6	5.5	3.2
	0.005*n*	4.9	0.9	+ 4.0	5.8	6.0	3.5
K_2SO_4	0.1*n*	4.0	2.2	+ 1.8	6.2	5.7	3.9
	0.05*n*	5.3	1.4	+ 3.9	6.7	5.8	3.7
	0.01*n*	0.4	0.2	+ 0.2	0.6	5.9	3.3
	0.005*n*	1.0	0.1	+ 0.9	1.1	5.8	3.3
$Ca(NO_3)_2$	0.1*n*	3.8	14.3	— 10.5	18.1	5.9	9.5
	0.05*n*	3.7	17.0	— 13.3	20.7	5.8	9.5
	0.01*n*	3.9	15.8	— 11.9	19.7	5.8	8.9
	0.005*n*	3.9	15.6	— 11.7	19.5	5.7	9.3

*) After removal of free carbonic acid.

sorption-character from NaCl and KCl-solutions as a function of the concentration employed is of particular interest. Lundegardh and collaborators did not find consistent results when working with dilute solutions [2], [7]) of chlorides, but established already a tendency for preferential anion-intake with increasing atomic weight of the cation [7]). Hoagland [3]), Niklewski [5]) and Kreyzi [1]) established an equivalent intake of KCl and explained this fact on the basis of equal speed-movement of the two ions and the equal permeability of the protoplasma for both ions. An equivalent char-

acter is also ascribed by Reed and Haas [6]) to the absorption of both ions from a NaCl solution. Contrary to the findings of these investigators Mengdehl and Pirschle [26]) found the absorption from a KCl solution to be a preferentially cationtic one.

As pointed out before the equal intake of anion and cation found by the a.m. authors has mainly to be ascribed to the weak concentrations employed. A further cause for the contradictions observed is to be found in the experimental technique employed, the plants being grown in complete nutrient solutions and only later transferred to a single salt solution for the purpose of the actual experiment. No doubt, that under these conditions the results obtained do not only refer to the influences to which the plant was subject during the actual experiment, but also to those of its earlier life-history.

With regard to the rate of absorption as dependent upon the cation, we see that the largest Cl-absorption was found in the case of KCl, followed by NaCl and the smallest absorption was found in the case of NH_4Cl.

Regarding the quantity of both ions absorbed from these three series the gradation is as follows:

$$K > Na > NH_4$$

The absorption from K_2SO_4 was weak. From $Ca(NO_3)_2$ the absorption was strong and must be related to the strong absorption of the anion.

Concerning the influence of the concentration of the solution on the rate of absorption, we found that the absolute quantities absorbed increase with the concentration; but when compared with the original concentration of the ions in the solution, there is a relative decrease in the rate of absorption.

3. *Changes in the hydrogen ion concentration caused by seedlings growing in various single-salt solutions.* pH determinations carried out at intervals throughout the experiment show fundamental changes in the hydrogen ion concentration of the original salt solution. The reason for these changes is the process of ion absorption from the salt solutions in which the plant is growing. The influence of the barley seedlings when grown in solution of K_2SO_4 and NH_4Cl was expressed by an increase in the hydrogen ion concentration. In contrast, in the case of $Ca(NO_3)_2$ there occurred an ap-

TABLE IV

Changes in the reaction of salt solutoins resulting from growing Barley seedlings therein

Solution	Concentration	pH value						Quantity of Bicarbonates in the solution
		In the original solution	After one day	After seven days	After fourteen days	After eighteen days *)		10^{-1} M.E. of HCO_3
						Sol. as found	Sol. after boiling	
KCl	0.1*n*	6.2	5.8	5.7	5.8	6.5	8.2	0.7
	0.05*n*	6.2	5.8	5.4	5.4	6.2	8.1	0.4
	0.01*n*	6.1	5.6	4.1	4.2	4.5	4.5	0
	0.005*n*	6.6	5.7	4.4	4.2	4.5	4.4	0
NaCl	0.1*n*	6.9	6.0	5.9	6.5	6.7	8.7	1.5
	0.05*n*	6.7	6.0	5.8	6.3	6.2	8.2	1.2
	0.01*n*	6.6	5.5	4.4	4.4	4.3	4.3	0
	0.005*n*	6.9	5.5	4.7	4.6	4.5	4.5	0
NH_4Cl	0.1*n*	5.5	5.3	4.7	4.6	4.5	4.4	0
	0.05*n*	5.4	5.3	4.9	4.8	4.7	4.6	0
	0.01*n*	5.5	4.8	3.6	3.2	3.2	3.2	0
	0.005*n*	6.0	4.8	3.7	3.6	3.5	3.5	0
K_2SO_4	0.1*n*	5.7	5.1	4.2	4.0	4.0	3.9	0
	0.05*n*	5.8	4.8	4.0	3.8	3.8	3.7	0
	0.01*n*	5.9	4.8	3.8	3.6	3.7	3.3	0
	0.005*n*	5.8	5.0	3.9	3.7	3.7	3.3	0
$Ca(NO_3)_2$	0.1*n*	5.9	6.2	6.8	6.9	7.2	9.5	8.9
	0.05*n*	5.8	6.0	6.9	7.0	7.3	9.5	6.5
	0.01*n*	5.8	6.3	7.0	6.9	7.2	8.9	7.4
	0.005*n*	5.7	5.8	7.0	6.9	7.4	9.3	5.0

*) The pH value was determined once in the solution as found and again after boiling in order to remove the carbonic acid.

preciable alkalinisation. In the case of KCl and NaCl the final reaction is determined by the concentration of the solution. In the case of concentrated solutions a trend towards alkalinisation occurs. Summarizing we can say that the changes occurring in the reaction of salt solutions resulting from the growing of barley seedlings therein are the reflection of the nature of the process of absorption; these changes not only reflect the direction of the absorption but also serve as additional confirmation for the analytical results. This is shown in the following way:

A strong cationtic absorption was found in the case of K_2SO_4 and NH_4Cl. Parallel herewith a rise in the hydrogen-ion concentration was found in the solution.

An aniontic absorption was found in the case of $Ca(NO_3)_2$ and parallel herewith an alkalinisation of the solution. In the case of KCl and NaCl, chemical analysis of the plant material showed a turning point in the character of absorption. The absorption is changed from an equivalent one in dilute solutions to a one-sidedly aniontic one in concentrated solutions. In all these cases bicarbonates were found. pH determinations in these solutions also showed a turning point. In concentrated solutions alkaline pH values are found after removal of the excess of carbonic acid. This carbonic acid, originating from respiration processes, has the function of a regulator for the reaction of the solution preventing extreme alkalinisation despite the one-sided aniontic absorption by the plant.

4. *The process of ion intake and outgo in salt solutions of 0.05n and the role of the respiration process in the preservation of the electrostatic equilibrium.* It is well known that ion absorption from a salt solution does not have an equivalent character for both ions in many cases. The plant reveals an elective power of absorption towards certain ions from among those at its disposal [28]). It may be assumed that the decisive factor is not only given by the physico-chemical properties of the ion, but also by this elective power of the plant. Unequal absorption of ions now gives rise to disturbances in the electrostatic equilibrium of the plant and its life-medium. The preservation of this equilibrium has to be accomplished by the plant itself. It is achieved both by exchange processes between plant and medium and by the activity of its respiration process.

First this former process, i.e. the exchange of mineral ions may be discussed. The results of a complete chemical analysis are given in Table V. They show again that the process of ion-absorption is not a one-directional one. It is, of course, well known that the process of ion-intake into the cell is accompanied by the reverse process, i.e. by the outgo of ions from the cell.

In our case the ions serving as exchange to take the place of cations absorbed, are mainly $K^{\cdot}$, $Ca^{\cdot\cdot}$ and $Mg^{\cdot\cdot}$. An outgo of Mg occurred in all the experiments, being especially strong in the case of NH_4Cl and of potassium salts. In the latter case, i.e. KCl and K_2SO_4, Mg

TABLE V

Absorption and exchange of ions by Barley seedlings growing in salt solutions of concentration 0.05*n*
(10^{-1} M.E. per gram of dry plant material)

Solution (0.05*n*)	Na	K	Ca	Mg	N	Cl	SO_4	HPO_4	Total Cations	Total Anions	Differ. between Ions	Total Ions
KCl	0.1	5.9	−0.8	−1.6	–	7.1	—	−0.7	3.6	6.4	−2.8	10.0
NaCl	4.0	−1.7	−0.1	−0.7	–	3.7	—	−0.4	1.5	3.3	−1.8	4.8
NH_4Cl	–	–	0.1	−1.6	5.4	2.6	—	−1.2	3.9	1.4	2.5	5.3
$Ca(NO_3)_2$	0.3	−0.4	2.6	−0.9	6.2	0.2	—	−0.7	1.6	5.7	−4.1	7.3
K_2SO_4	0.2	9.3	−0.5	−1.2	–	–	2.8	−0.2	7.8	2.6	5.2	10.4

Quantity of bicarbonates in the solution

Solution (0.05*n*)	HCO_3 M.E. 10^{-1}
KCl	2.5
NaCl	1.8
NH_4Cl	—
$Ca(NO_3)_2$	4.3
K_2SO_4	—

pH values of the solutions

Beginning of experiment	End of experiment	
	Solution as found	After boiling
6.5	5.9	8.2
6.8	5.7	9.2
5.4	4.7	4.8
5.6	7.1	9.6
6.0	4.1	4.0

is joined by Ca. An anologous phenomenon of Ca-outgo in experiments with K salts was found by Hoagland [3]) and Niklewski [5]). In their opinion $Ca^{\cdot\cdot}$ is the principal ion exchanged for $K^{\cdot}$. In our experiments with NaCl a strong outgo of K and a weak one for Ca and Mg was observed. An outgo of K-ions was also found in the experiments with $Ca(NO_3)_2$. In the case of NH_4Cl no outgo of Na and K did take place. In all experiments an outgo of phosphoric acid was observed, a fact which corresponds to observations made by Lundegardh [18]) and Niklewski [5]). This outgo was stronger in the case of Cl absorption than in the case of SO_4.

But this ion-exchange is in itself not sufficient to preserve the

electrostatic equilibrium as will readily be observed by the figures given in table V. It has already been stressed by various authors [2 5], [17], [18], [26], [27], [29] that in many cases the electrostatic equilibrium can only be preserved by the respiratory activity of the plant yielding HCO_3-, H-, and OH-ions. Our figures (Tables IV and V) confirm this view and give a complete quantitative summary of the processes involved.

A one-sided intake of cations (NH_4Cl and K_2SO_4) is coupled with the appearance of H-ions in the solution. A rise in the acidity of the life-medium of the plant is taking place and no HCO_3-ions are found. Not so in the case of a one-sided anion absorption (NaCl, KCl, $Ca(NO_3)_2$). Here bicarbonates are found in the solution and their amount balances the difference between the sum-total of anions and cations. The life-medium shows a trend towards alkalinity, although in the case of NaCl and KCl this trend becomes apparent only after the removal of the excess of CO_2.

The fact that one-sided aniontic absorption and a corresponding trend of alkalinisation has been found in relatively concentrated solutions of NaCl may possibly prove to be of great ecological importance. If this phenomenon will prove to be true generally, it may constitute a contributing factor wereever alkalinisation and soda formation is concerned.

Summary

1) Barley seedlings grown in single salt solutions showed a one-sided cation absorption in the case of NH_4Cl and K_2SO_4 with corresponding rise in acidity.

2) In the case of $Ca(NO_3)_2$ a one-sided intake of anions occurred.

3) In the case of NaCl and KCl, intake of both ions was equivalent in low concentrations. With higher concentrations the intake was decidedly aniontic.

4) In all cases where the intake of anions exceeded the intake of cations, the electrostatic equilibrium was provided by the respiratory process of the plant, yielding HCO_3-ions.

5) This production of bicarbonate-ions has a marked influence on the milieu, constituting a trend towards alkalinisation.

6) It is suggested that the one-sided anion-absorption in the case of NaCl and the corresponding formation of bicarbonate in the milieu may constitute a contributing factor for alkalinisation and soda-formation in the soil.

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IMPORTANCE OF MOLYBDENUM IN THE NITROGEN METABOLISM OF MICROORGANISMS AND HIGHER PLANTS

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Introduction. The biological importance of molybdenum was first demonstrated by Bortels[10]) in 1930 in experiments with the free-living nitrogen-fixing bacterium *Azotobacter chroococcum.* In a synthetic medium without combined nitrogen this organism grew poorly, unless small amounts of molybdenum were added to the nutrient solution. Apparently this element is indispensable for the assimilation of the gaseous nitrogen. Other investigators have since confirmed this observation (Burk and Lineweaver[14]), Birch-Hirschfeld[8]), Schröder[27]), Kluyver and Reenen[20]), Krzemieniewsky and Kovats[21]), Burema and Wieringa[17]).

Not only *Azotobacter* but *Clostridium pasteurianum* as well requires traces of molybdenum when growing without combined nitrogen[11]).

As for the nitrogen fixation by leguminous plants there is some evidence that, in this process too, molybdenum is an important element (Bortels[12]), Bertrand[7]), Bobko and Savvina[9])). These authors, however, merely reported a more or less stimulating effect of molybdenum on the growth of leguminous plants and Hoagland[18]), reviewing the literature on this subject, came to the conclusion that ,,the few experiments now on record certainly do not provide decisive, or even very strong, evidence, that molybdenum is indispensable for fixation of nitrogen by leguminous plants".

In Australia and New Zealand a striking response to molybdenum was found with alfalfa and subterranean clover growing in

*) Agronomist of the ,,Staatsmijnen in Limburg".

acid ironstone soils (personal communications of Miss Margaret Thomas, Adelaïde, South Australia and Dr. A. C. S. Wright, Wellington, New Zealand). See also Anderson[2]) and Jensen and Betty[19]), cited by Hoagland[18]), and Anderson *et al.*[3]). The fact that the legumes gave only insignificant responses to molybdenum when sufficient nitrate or ammonium nitrogen was provided, whereas grasses grown alone on the same soil responded to combined nitrogen but not to molybdenum, shows that molybdenum stimulated the fixation of gaseous nitrogen by the leguminous plants growing on these soils.

In 1936 Steinberg[28]) showed that *Aspergillus niger* requires small amounts of molybdenum when grown in a medium with nitrate as the sole source of nitrogen. With ammonium nitrogen the response to molybdenum was found to be considerably less.

The essentiality of traces of molybdenum for the normal development of higher plants was first demonstrated by Arnon and Stout[6]) in 1939 in waterculture experiments with tomato plants. Grown in a rigidly purified nutrient solution, containing all of the nutrient elements regarded as essential, (N, P, K, Ca, Mg, S, Fe, B, Mn, Z and Cu), characteristic deficiency symptoms became apparent in a few weeks. The lower leaves developed a mottling and in later stages necrosis at the margins and an involution of the laminae was shown. Almost all of the blossoms abcissed without setting fruit. With 10 γ of molybdenum added to 1 l of nutrient solution normal growth was obtained. Of 19 other elements tested, (Ti, V, Cr, W, Co, Ni, Al, As, Cd, Sr, Mg, Pb, Li, Rb, Br, I, F, Se and Be), none was capable of replacing molybdenum. No details concerning the composition of the nutrient solution and the nitrogen compound used in these experiments were given. In two other series of experiments with barley [4]) and with lettuce and asparagus [5]) respectively, some evidence was obtained that growth improved after the addition of molybdenum. In the first-mentioned experiments this happened when the nitrogen was present as an ammonium salt.

Hoagland[18]) in culture solution experiments with plum seedlings observed qualitatively a response to molybdenum. The leaves of the molybdenum-deficient plants were dwarfed; some developed a diffuse mottling and many showed light brown areas of dead tissue at the tips and margins.

Piper[25]) found that molybdenum was essential for the normal growth of oat plants. His nutrient solutions were prepared with specially purified nutrient salts. In the absence of molybdenum necrotic areas appeared on the leaves. At first these leaves turned backwards in a sharp but smooth bend, but later a kink developed at the collapsed area and the midportion of the leaf dried out to a light reddish-brown colour. The yields of grain but not total yield were influenced by molybdenum.

Warington[30]) observed a marked beneficial effect of the addition of small amounts of molybdenum on both yield and appearance of lettuce grown in nutrient solutions.

In the authors previous experiments[23]), [24]) on the influence of copper on the growth of *Aspergillus niger* a comparison was made of two methods of purification of the nutrient medium: adsorption on charcoal and recrystallization of the nutrient salts and glucose. Upon addition of copper impaired growth of *Aspergillus* was still obtained in the medium purified by recrystallization. Obviously, the medium purified with charcoal, though free from copper, contained some substance which was removed by recrystallization of the nutrient salts and the sugar. The results of Steinberg[29]) suggested that molybdenum deficiency was the cause of the poor growth of *Aspergillus* in the medium purified by recrystallization. Another set of experiments was therefore started with recrystallized salts, this time with a trace of molybdenum added to some of the cultures. When molybdenum was added a normal development of *Aspergillus niger* took place, thus confirming the results of Steinberg that molybdenum is an indispensable element for this organism.

The purpose of the present investigation was to study the biological significance of molybdenum for both microorganisms and higher plants with special reference to the effect of varying concentrations on growth and nitrogen metabolism.

Experimental methods. Culture solutions were used in the experiments with microorganisms as well as in those with higher plants. In preparing these solutions water distilled in glassware was used throughout. Salts free or practically free from molybdenum were selected in the preparation of the nutrient media. A biological assay, described below, was employed to test the different brands of salts for molybdenum. This method appeared to be of

great value in preparing solutions practically free from molybdenum.

The culture solutions employed for the experiments with tomato, barley, oat and pea plants were generally not aerated in order to prevent any contamination with traces of molybdenum. An improved air supply, especially important in the experiments with peas, was obtained by only half filling the culture vessels with nutrient solution.

Experiments with Aspergillus niger. a. Effect of molybdenum concentration on growth. The following nutrient solution was used for growing *Aspergillus*:

H_2O	1 l	$Na_2CO_3.10H_2O$	0.25 mg
Glucose	50 g	$FeCl_3.6H_2O$	3.00 mg
KNO_3	5 g	$MnSO_4.4H_2O$	0.30 mg
K_2HPO_4	0.6 g	$ZnSO_4.7H_2O$	2.00 mg
$MgSO_4.7H_2O$	0.6 g	$CuSO_4.5H_2O$	0.60 mg
$CaSO_4.2H_2O$	0.5 g		

50 cc portions of this solution were pipetted into 500 cc Erlenmeyer flasks, autoclaved for 10 minutes at 105°C, inoculated with a spore suspension of *Aspergillus*, strain M, and incubated for 4 days at 31°C. Molybdenum was added in increasing amounts. The basic source of nitrogen was nitrate but some flasks were provided with an additional quantity of $(NH_4)_2SO_4$ *). Table 1 gives the yields of dry mycelium.

TABLE 1

Effect of increasing amounts of molybdenum on the growth of *Aspergillus niger*

$Na_2MoO_4.2H_2O$ added per flask γ	Yield †) (dry weight) g
0	0.187
0 §)	0.765
0.01	0.562
0.05	0.901
0.10	0.946
1.00	0.951
10.00	1.020
10.00 §)	1.148

§) 25 mg of $(NH_4)_2SO_4$ per flask added.

†) average of duplicate values.

*) This salt was recrystallized two times from glass distilled water.

From these data it is seen that the addition of very small amounts of molybdenum permitted good development of *Aspergillus*. Ammonium nitrogen had a marked effect on the molybdenum requirement of the fungus, as was observed by Steinberg [29]).

Since it was intended to use the growth rate curve of *Aspergillus* for the biological estimation of small amounts of molybdenum, the above-mentioned experiment was repeated with quantities of Na_2Mo_4O smaller than 0.01 γ. Some cultures with traces of ammonium nitrogen were included to determine whether molybdenum could be estimated by *Aspergillus* in materials containing some ammonia, as for instance, soil †). The results of this experiment are given in table II.

TABLE II

Effect of increasing amounts of molybdenum on the yield of *Aspergillus niger*

$Na_2MoO_4.2H_2O$ per 50 cc of nutrient sol. γ	NH_4–N per 50 cc of nutrient sol. γ	yield *), dry weight g	sporulation	appearance of mycelium
0	0	0.165	0	entirely mucous
0	20	0.171	0	,, ,,
0	200	0.211	0	,, ,,
0.001	0	0.253	0	,, ,,
0.0025	0	0.294	0	partially ,,
0.005	0	0.450	0	,, ,,
0.010	0	0.558	0	,, ,,
0.025	0	0.797	scant	normal
0.050	0	0.868	normal	normal
0.100	0	0.900	normal	normal

*) average of duplicate values.

The data of table II are plotted in fig. 1. This curve as well as the difference in sporulation with different amounts of molybdenum may be used for the biological determination of minute quantities of molybdenum in various materials, as will be described below.

b. Effect of nitrate and ammonium nitrogen on the molybdenum requirement of *Aspergillus niger*. In order to elucidate the influence of ammonium nitrogen on the molybdenum requirement a series of experiments was undertaken to test the following possibilities:

†) Vanadium which partly can replace molybdenum in the fixation of gaseous nitrogen by *Azotobacter* had no influence on the growth of *Aspergillus niger* when added in an amount of 0.1 γ $NaVO_3$ per 50 cc of molybdenum-free nutrient solution.

1. Ammonium salts favour, whereas nitrates depress the absorption of the trace of molybdenum unavoidably present in every nutrient medium as an impurity. If this assumption were correct then cultures with $(NH_4)_2SO_4 + KNO_3$ should have made less growth than those with the ammonium salt only. No effect was observed, however, from the addition of potassium nitrate.

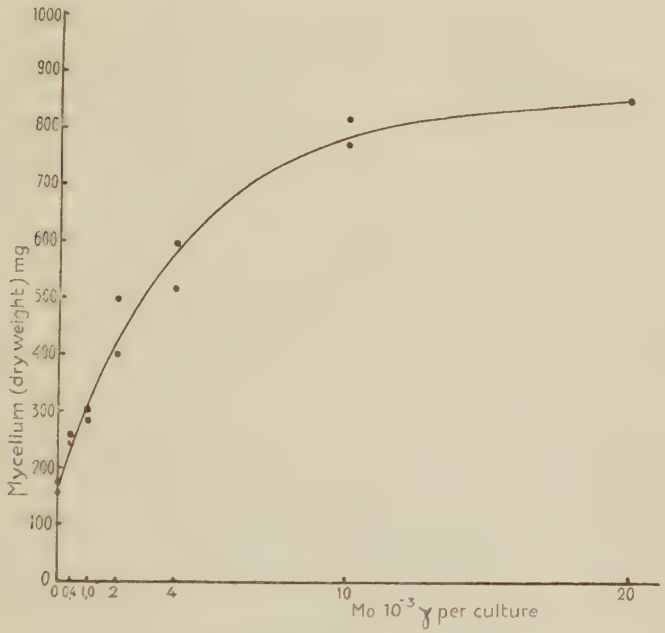

Fig. 1. Effect of molybdenum on the growth of *Aspergillus niger* in a nutrient solution with nitrate nitrogen.

2. The less acid reaction of the nutrient medium following the absorption of nitrate nitrogen depresses the absorption of molybdenum. 1 γ of Na_2MoO_4 was added to two-day and three-day old molybdenum-deficient nitrate cultures. Although the growth of these mycelia was poor and their appearance mucous, a response to molybdenum was seen twelve hours after the addition of this element. 30 hours later the appearance of these cultures was quite normal with an abundant formation of black spores. Thus the reaction of the nitrate-containing medium was no hindrance to the absorption of newly introduced molybdenum.

3. The ammonium salts are less pure than the nitrates. To test

this possibility an excess of molybdenum-free sodium carbonate was added to a solution of the ammonium salt. The ammonium was distilled and absorbed in a solution of molybdenum-free KH_2PO_4. The residue, which should have contained the impurities, and the distillate were separately tested for molybdenum with *Aspergillus*. Table III contains the results.

TABLE III

Effect of distilled ammonium nitrogen and residue of ammonium sulfate on the growth of *Aspergillus niger* in a molybdenum-deficient nitrate medium

Nitrogen source	Addition	$Na_2MoO_4.2H_2O$ added, γ	yield *) of *Aspergillus*, g
KNO_3	Control	0	0.134
,,	$KH_2PO_4 + Na_2CO_3$	0	0.241
,,	$KH_2PO_4 + Na_2CO_3$	1	0.892
,,	residue of distilled $(NH_4)_2SO_4$	0	0.175
,,	,, ,, $(NH_4)_2SO_4$	1	0.788
,,	distilled NH_3, absorbed in KH_2PO_4	0	0.786
,,	,, NH_3, ,, KH_2PO_4	1	0.855
$(NH_4)_2SO_4$	Control	0	0.810
,,	Control	1	0.900

*) average of duplicate values

These data clearly show that the beneficial effect of the ammonium salt has to be attributed to the ammonium nitrogen as such and not to a molybdenum impurity. This could be demonstrated in another way. *Aspergillus niger* was grown in a series of ammonium and nitrate media with and without molybdenum. At the conclusion of the growing period the mycelia were weighed, ashed and the ash added to the nutrient solution of a new series of molybdenum-deficient cultures supplied with nitrate nitrogen only. The results are given in table IV.

TABLE IV

Effect of adding ashed mycelia to molybdenum-deficient *Aspergillus* cultures supplied with nitrate nitrogen only

History of ashed mycelia	Dry weight (g) of *Aspergillus* *), supplied with ash
KNO_3, no Mo, Dr. wt. *) 0.351 g	0.297
KNO_3, 1 γ of Na_2MoO_4 per cult. ,, ,, 0.776 g	0.797
$(NH_4)_2SO_4$, no Mo ,, ,, 0.778 g	0.265
$(NH_4)_2SO_4$, 1 γ of Na_2MoO_4 per cult. ,, ,, 0.771 g	0.814
distilled NH_4, no Mo ,, ,, 0.786 g	0.276
distilled NH_4, 1 γ of Na_2MoO_4 per cult. ,, ,, 0.855 g	0.751

*) average of duplicate values.

It can be seen that the ash of the nitrate and ammonium mycelia gave similarly poor growth of *Aspergillus* in a molybdenum-free nitrate medium, thus indicating that the heavier yields of the ammonium mycelia contained as little molybdenum as the nitrate ones.

The conclusion which must be drawn from all of these data is, that in a medium with nitrate nitrogen *Aspergillus* requires a higher molybdenum supply than in a solution with an ammonium salt.

The question arises to what extent the poor growth of the fungus in the molybdenum-deficient nitrate medium is caused by an insufficient reduction of the nitrate to ammonia, i.e. by a lack of available nitrogen. Steinberg[29]), who also discusses this question, is of the opinion that nitrogen and molybdenum deficiency are quite similar. However, molybdenum-deficient mycelia are mucous and free of spores. On the other hand mycelia with an insufficient nitrate supply do not show this mucous type of growth and develop relatively abundant spores.

More details concerning the influence of molybdenum on nitrate reduction will be recorded in the paragraphs on the experiments with denitrifying bacteria and higher plants.

c. Estimation of molybdenum with *Aspergillus niger*. The fact that increasing amounts of molybdenum induce increasing yields of mycelium and sporulation of *Aspergillus niger* suggests the use of this organism as a biological test for the estimation of molybdenum. To determine plant-available molybdenum in soil, small amounts of air dried soil were added to 50 cc portions of molybdenum-free nutrient solution in 500 cc Erlenmeyer flasks, previously sterilized for 5 minutes at 105° C. After inoculation with a suspension of *Aspergillus* spores and incubation at 31° C for four days the amount of available molybdenum was estimated by comparing the yield and the spore formation of the culture with a set of standards grown with known amounts of molybdenum.

In one experiment three soils of different fertility were compared: a clay soil, a garden soil, both of high fertility, and a poor, sandy soil, low in humus. In these cases 500 mg of air-dried soil were added to each flask. This amount appeared to be too high: normal yields and spore formation were obtained, showing that the content of available molybdenum was higher than 0.025 γ in 500 mg of soil.

In a second experiment three different soils were compared in por-

tions of 100 mg of air-dried soil per flask. Amounts of 0.015, 0.02, and 0.10 γ per gram of soil were found *).

For the estimation of total molybdenum in plant materials 10 mg of dried, ground tissue were ashed and the ash used in the *Aspergillus* test. Table XVI contains some results.

Experiments with denitrifying bacteria. If traces of molybdenum are essential for the reduction of nitrate, this element should prove indispensable for the denitrifying bacteria, which when grown anaerobically depend on nitrates for the oxygen used in oxidizing energy-yielding substrates. This assumption was tested as follows. A denitrifying bacterium, strain S, was isolated from a garden soil, and grown in a synthetic medium of the following composition:

H_2O	1 l		
Glucose	2.5 g	$FeCl_3.6H_2O$	0.5 mg
KNO_3	1.0 g	$MnSO_4.4H_2O$	0.1 mg
K_2HPO_4	1.0 g	$ZnSO_4.7H_2O$	0.1 mg
$MgSO_4.7H_2O$	0.5 g	$CuSO_4.5H_2O$	0.1 mg
$Na_2CO_3.10H_2O$	0.2 mg		

An extract of ground *Aspergillus* mycelia grown in a molybdenum-free nutrient solution was also added.

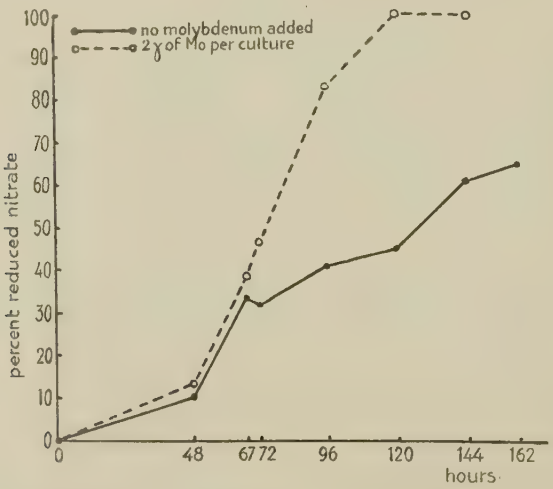

Fig. 2. Effect of molybdenum on denitrification.

*) Ter Meulen [22]) determined molybdenum in many plant tissues and in a number of soils.

The bacteria were incubated at 25°C in 50 cc stoppered bottles, without and with 5 γ of $Na_2MoO_4.2H_2O$ added. After different periods of incubation one bottle with and one without molybdenum were analysed for nitrate according to the xylenol-method [1]).

The results are shown in fig. 2. It is seen that with molybdenum denitrification proceeded more intensively than in the absence of this element.

In a second series of experiments four strains of denitrifying bacteria were used. Extract of molybdenum-free *Aspergillus* mycelia was added to one set of bottles only. Nitrate and nitrite †) determinations were carried out after five and ten days of incubation at 25 C respectively. Tables V and VI contain the results of these experiments.

TABLE V

Effect of molybdenum on denitrification (incubation time 5 days)

Bact. strain	$Na_2MoO_4.2H_2O$ per bottle, γ	Extract of *Asp. niger* added	After five days incubation at 25°	
			NO_3–N disappeared *), mg	NO_2–N per bottle, mg
W 1	0	0	2.7	1.3
,,	0	+	1.7	1.7
,,	5	0	9.6	7.9
,,	5	+	9.3	8.7
S 1	0	0	0.0	0.0
,,	5	0	12.6	6.0
S 2	0	0	0.0	0.0
,,	0	+	0.2	0.0
,,	5	0	10.8	5.0
,,	5	+	9.8	9.4
W 2	0	0	0.7	0.0
,,	0	+	2.3	0.0
,,	5	0	6.2	2.7
,,	5	+	9.2	7.8

*) Initial nitrate concentration: 13.1 mg N per bottle (50 cc).

These data reveal quite clearly that molybdenum is indispensable for the denitrification process. With two strains of bacteria, S 1 and S 2, no growth at all was obtained in the absence of molybdenum. Obviously the nitrate could not be used as an oxygen supply. Strain W 1, which only reduces nitrate to nitrite, showed an insignificant reduction of nitrate when molybdenum was omitted

†) According to the Griess–Romijn-method (26).

TABLE VI

Effect of molybdenum on denitrification †) (incubation time 10 days)

Bact. strain	$Na_2MoO_4.2H_2O$ per bottle, γ	After 10 days incubation at 25°C	
		NO_3–N disappeared *), mg	NO_2–N per bottle, mg
W 1	0	1.4	1.7
,,	5	9.3	8.5
S 1	0	4.5	0.0
,,	5	13.1	0.0
S 2	0	0.0	0.0
,,	5	10.2	8.1
W 2	0	0.0	0.0
,,	5	8.0	7.4

†) No extract of *Aspergillus* was added to these cultures.
*) Initial nitrate concentration: 13.1 mg N per bottle (50 cc).

from the nutrient solution. With this strain, as well as with strain W 2, the effect of molybdenum was very conspicuous.

In a preliminary experiment with strain S 1 no effect of molybdenum on the *oxidation-reduction potentials* of denitrifying cultures was observed during the first four days of incubation. After that the cultures with molybdenum had a lower E_h than those deficient in this element. From nitrate determinations it was concluded that these differences were brought about by the fact that in the + Mo-cultures the nitrate had been consumed whereas in the absence of molybdenum this salt was still present.

Experiments with higher plants. In the years 1942 and 1943 water-culture experiments were carried out with tomato, barley and oat plants. In the barley and tomato experiments of 1942 a nutrient solution of the following composition was used

H_2O	1 l	$Na_2CO_3.10H_2O$	1.25 mg
KNO_3	0.35 g	$FeCl_3.6H_2O$	25.— mg
$(NH_4)_2SO_4$	0.15 g	$MnSO_4.4H_2O$	1.— mg
K_2HPO_4	0.125 g	$ZnSO_4.7H_2O$	0.25 mg
KH_2PO_4	0.200 g	$CuSO_4.5H_2O$	0.25 mg
$MgSO_4.7H_2O$	0.25 g	H_3BO_3	0.25 mg
$CaSO_4.2H_2O$	0.25 g		

Only those salts were applied, which in experiments with *Aspergillus* were found to be practically free from molybdenum. The seeds were germinated in quartz sand, previously treated with nitric acid.

a. Experiments with tomato plants. Two plants were placed in each glass jar containing 1.2 l of culture solution. When molybdenum was omitted from this solution very pronounced deficiency symptoms were observed. First a mottling of the leaves occurred which after some time changed to an almost yellow shade. The growth of the whole plant decreased and soon stopped entirely. Necrotic spots appeared in the yellow tissue, especially along the margins of the upper part of the leaflets and many older leaves

Fig. 3. Effect of molybdenum on tomato plants (left without, right with 100 γ of Na_2MoO_4 per culture).

died. In many cases a characteristic involution of the leaflets occurred (see fig. 3). The plants were harvested at an early stage. The yields are given in table VII.

TABLE VII

Effect of molybdenum on the yield of tomato plants in a nutrient solution

$Na_2MoO_4.2H_2O$ per culture, γ	Leaves + stem dry weight, g	Roots, dry weight, g	Remarks
0	6.45	0.77	pronounced deficiency symptoms
0	4.77	0.60	,, ,, ,,
0 *)	13.40	2.37	light ,, ,,
,, *)	13.12	2.32	,, ,, ,,
100 †)	18.37	3.03	normal plants

*) probably contaminated with traces of molybdenum.

†) added after every change of the culture solution.

In 1943 another experiment with tomato plants was carried out. The nutrient solution used, differed only slightly from the previous one: KH_2PO_4 and K_2HPO_4 were added in amounts of 0.25 and 0.07 g per 1 respectively, whereas nitrogen was supplied as KNO_3 only. The first symptoms of molybdenum deficiency were observed after 3 weeks. Two months later when the greater part of the lower half of the plants had died down, they were harvested, dried and weighed (table VIII).

TABLE VIII

Effect of molybdenum on the yield of tomato plants			
$Na_2MoO_4.2H_2O$ per culture, γ	Leaves + stems dry weight, g	Roots, dry weight, g	Remarks
0	5.85	0.70	no fruits formed
0	7.42	1.01	,, ,, ,,
0	10.67	1.89	probably some contamination
0	10.90	1.90	with molybdenum; fruit formation rather good
100 *)	20.60	3.93	normal fruit-setting
100 *)	20.25	2.65	,, ,, ,,

*) added after every change of the culture solution.

b. Experiments with barley. In barley molybdenum deficiency symptoms appeared when the plants were about four weeks old. The leaves became light green in colour and in the youngest ones a chlorotic striation was seen. With 100 γ of Na_2MoO_4 added per jar the plants had a dark green colour. Some weeks later in the absence of molybdenum the ends of the leaves had died. The ears emerged slowly and showed a yellowish-green shade. The seed production was poor (table IX). The yield of straw was somewhat higher in the absence of molybdenum, due to the emergence of young green shoots.

TABLE IX

Effect of molybdenum on the yield of barley in nutrient solution *)							
$Na_2MoO_4.2H_2O$ per culture, γ	Grains		Straw, dry weight, g	Roots, dry weight, g	Number of ears per jar.	
	dry weight g	number			normal	green
0	1.79 ± 0.46	52 ± 12	10.10 ± 0.74	1.36 ± 0.08	6 ± 0.5	9 ± 1.6
100 †)	5.77 ± 0.38	135 ± 8	8.42 ± 0.86	1.49 ± 0.05	8 ± 0.7	3 ± 1.1

*) average values of four cultures.

†) added after every change of the culture solution.

c. Experiments with oats (1943). In this experiment a nutrient solution of a similar composition as in the experiment with tomato plants in the same year was employed.

In the absence of molybdenum the ripening of the plants was much retarded. The emergence of young green shoots was again observed. The results are presented in table X.

TABLE X

Effect of molybdenum on the yield of oats in nutrient solution *)

$Na_2MoO_4.2H_2O$ per culture, γ	Grains		Straw, dry	Roots, dry
	dry weight, g	number	weight, g	weight, g
0	3.54 ± 0.44	137 ± 13	12.22 ± 0.78	1.36 ± 0.10
100 †)	5.98 ± 0.45	240 ± 26	12.82 ± 0.66	1.36 ± 0.74

*) average value of four, (0 Mo), and three cultures, (+ Mo), respectively.
†) added after every change of the culture solution.

d. Importance of molybdenum in the nitrate reduction of green plants. In order to know whether or not molybdenum is essential for the nitrate reduction in green plants, the following experiments with barley and tomato plants were carried out. Young plants of both species were transplanted into glass jars containing a nutrient solution without molybdenum and with a moderate supply of nitrate nitrogen. The experiment with barley plants consisted of two series of eight jars each, one series without and one with 100 γ of Na_2MoO_4 per jar. After two weeks the nutrient solution was replaced by a solution without nitrogen. Nine days later when the nitrate in the plants was depleted, 0.75 g of potassium nitrate was added to each jar. After six days all of the plants were harvested and analysed for protein *) and nitrate nitrogen. The plants without molybdenum had a somewhat pale green colour, while those provided with traces of this element were dark green. The results of the analyses are given in table XI.

These data show that molybdenum-deficient plants contain more nitrate and less protein nitrogen than those provided with this element. This fact was more clearly shown in an experiment with tomato plants which was carried out in August and September 1944. As in the experiment with barley, the tomato plants were first

*) The proteins were precipitated in the ground fresh tissue with trichloroacetic acid.

grown in a nutrient solution with a moderate supply of nitrate and without molybdenum. When the plants had grown for about five weeks in this medium the first symptom of molybdenum deficiency, a slight mottling, was observed. In addition slight symptoms of nitrogen deficiency became apparent. Of the fourteen jars used, each

TABLE XI

Effect of molybdenum on the nitrate reduction in barley plants

$Na_2MoO_4 . 2H_2O$ added, γ	Leaves + stems			Roots	
	fresh weight g	protein-N mg	nitrate-N mg	fresh weight g	nitrate-N mg
0 *)	20.0 ± 0.60	62.4 ± 1.96	16.8 ± 0.58	9.6 ± 0.91	4.3 ± 0.48
200 †)	21.7 ± 0.40	71.5 ± 1.73	11.3 ± 0.83	7.9 ± 0.18	3.2 ± 0.16

*) average values of six separately analysed cultures.
†) ,, ,, ,, eight ,, ,, ,,

containing one tomato plant, two were harvested and analysed for different nitrogen fractions. The other twelve were divided into two series of plants resembling each other as much as possible. The nutrient solution of all of these cultures was changed. The nitrate concentration was raised to 1 g of potassium nitrate per culture. One group of plants received 200 γ of $Na_2MoO_4 . 2H_2O$ per jar, the other group remained without molybdenum. Three days later, when the first colour differences between the two groups of plants were observed, they were harvested and the fresh leaves, stems and roots analysed for protein, soluble organic nitrogen and nitrate (table XII).

The results of this experiment clearly show that molybdenum is indispensable for the nitrate reduction in tomato plants. In the absence of this element nitrate is accumulated in the leaves. Soluble organic nitrogen compounds and proteins are practically not formed. In the stems the nitrate differences are less pronounced, whereas in the roots practically no differences are shown. Apparently in tomato plants the nitrate reduction takes place mainly in the leaves and the stems.

In order to prove that molybdenum is essential for the first step of the nitrate assimilation, i.e. the reduction of nitrate to ammonia, an experiment was carried out in which molybdenum- and nitrogen-deficient tomato plants were provided with nitrate and with ammonium sulphate respectively. Some weeks after the addition of the different nitrogen compounds great differences between the plants

TABLE XII

Effect of molybdenum on the nitrate reduction in tomato plants

$Na_2MoO_4 . 2H_2O$ added, %	Leaves				Stems				Roots			
	Fresh weight, g	Protein-N, mg	Sol. org. N, mg	NO_3-N mg	Fresh weight, g	Protein-N, mg	Sol. org. N, mg	NO_3-N mg	Fresh weight, g	Protein-N, mg	Sol. org. N, mg	NO_3-N mg
0 *)	11.30 †)	15.5	3.85	0.00	—	8.09	4.09	0.00	2.75	6.23	2.52	0.00
0 *)	11.72 †)	14.7	4.58	0.00	—	7.41	3.93	0.00	2.74	—	—	—
0 **)	5.08 ± 0.41	15.3 ± 0.5	3.42 ± 0.2	4.19 ± 0.48	7.54 ± 0.82	6.74 ± 0.80	3.79 ± 0.42	3.53 ± 0.36	3.34 ± 0.88	5.26 ± 0.57	2.62 ± 0.35	1.41 ± 0.46
200 **)	6.10 ± 0.25	19.1 ± 0.93	3.05 ± 0.25	0.61 ± 0.18	8.61 ± 0.71	8.63 ± 1.16	6.20 ± 0.66	2.70 ± 0.30	3.61 ± 0.15	4.92 ± 0.41	3.12 ± 0.55	1.32 ± 0.18

*) plants harvested before adding the nitrate.

†) fresh weight of leaves + stem.

**) average values of six seperately analysed plants.

were found. Those provided with nitrate showed yellow-green leaves with upwards curling margins. The addition of ammonium nitrogen, however, had resulted in the formation of dark green leaves, indicating an intensive nitrogen assimilation. The plants were harvested and analysed for some nitrogen fractions (table XIII).

TABLE XIII

Effect of molybdenum on the assimilation of nitrate and ammonium nitrogen

N-compound added	$Na_2MoO_4.2H_2O$ γ per jar	Leaves				Stems				Roots
		Fresh weight g	Protein-N *)	Sol. org. N *)	NO_3-N *)	Fresh weight g	Protein-N *)	Sol.-org. N *)	NO_3-N *)	Fresh weight g
KNO_3	0	19.38	— †)	7.9	13.77	27.50	6.3	4.6	9.58	6.60
,,	0	16.47	— †)	7.7	14.19	24.21	7.4	4.7	9.57	6.50
,,	200	17.75	21.7	9.9	0.41	33.35	9.1	5.4	3.61	13.55
,,	200	17.75	21.6	10.6	0.49	29.30	9.1	6.0	3.49	10.50
$(NH_4)_2SO_4$	0	10.50	31.5	10.6	1.34	15.35	10.7	10.7	2.60	5.40
,,	0	10.26	32.0	10.5	1.08	18.90	10.2	10.7	2.60	5.65

*) mg per 10 g of fresh weight.

†) both samples lost.

Although the total growth of the ammonium plants was less than that of the nitrate plants the table shows the following:

1. In the absence of molybdenum the nitrate content of the leaves is more than thirty times and that of the stems almost three times higher than that of the plants supplied with this element.

2. The content of the organic nitrogen fractions in leaves as well as in stems of the ammonia plants without molybdenum is even higher than that of the nitrate plants with molybdenum added.

This clearly shows that without molybdenum formation of organic nitrogen compounds from ammonia can take place.

Although it is possible that molybdenum is required for some other reaction in the plant life, one of the main functions is that of catalysing the nitrate reduction. This is not only true for the green plant but also for such microorganisms as *Aspergillus niger* and denitrifying bacteria.

The importance of molybdenum in the fixation of gaseous nitrogen. *a.* Experiments with *Azotobacter chroococcum.* The question whether molybdenum is an essential element for the growth of *Azotobacter* or for the nitrogen fixation of this organism only,

is dealt with by some authors. Birch–Hirschfeld[8]), cultivating *Azotobacter* in an oxygen-hydrogen atmosphere with nitrate as a nitrogen source, could not observe a respond to molybdenum. Burk[15]) first came to the conclusion that molybdenum is required for the N_2-fixation only; in media with nitrate, nitrite and asparagine no favourable effect of the addition of this element could be found. In a later report, however, this same author[16]) contends that molybdenum stimulates the growth of *Azotobacter*, when the nitrogen is added to the nutrient media as nitrate or asparagine.

Bortels[13]) found a highly stimulating effect of traces of molybdenum on *Azotobacter chroococcum* when this organism was grown in a nitrate medium. The concentration of molybdenum essential for optimal growth was found to be much smaller than when grown without combined nitrogen. With ammonium sulphate and asparagine a beneficial effect of molybdenum was also observed. In the absence of molybdenum far better results were obtained with ammonium than with nitrate nitrogen.

Burema and Wieringa[17]) also found that *Azotobacter chroococcum* requires less molybdenum in a nitrate medium than when grown without combined nitrogen.

The relation between molybdenum and nitrogen nutrition was investigated in the following experiment.

Azotobacter was grown in a medium of the following composition:

H_2O	1 l	$Na_2CO_3.10H_2O$	5 mg
Glucose	40 g	$FeCl_3.6H_2O$	25 mg
K_2HPO_4	2 g	$MnSO_4.4H_2O$	2 mg
$MgSO_4.7H_2O$	1 g	$ZnSO_4.6H_2O$	5 mg
$CaCO_3$	4 g	$CuSO_4.5H_2O$	4 mg

Erlenmeyer flasks of 100 cc capacity, containing 15 cc of nutrient solution, served as culture vessels. One set of flasks was supplied with 10 mg of nitrogen per flask as potassium nitrate, another set with 10 mg of nitrogen as ammonium sulphate, whereas a third one was given no combined nitrogen. No precautions were taken to exclude the N_2 of the air from the nitrate and ammonium cultures, as it was expected that no fixation of gaseous nitrogen would occur when combined nitrogen was present.

Molybdenum was given in amounts of 0, 0.05, 0.1, 0.2, 0.5, 1, 2,

5, 10 and 20 γ of $Na_2MoO_4.2H_2O$ per flask. Each flask was inoculated with five drops of a suspension of *Azotobacter chroococcum*. After eight days of incubation at 25°C differences were seen between the three sets of cultures. Without combined nitrogen practically no growth occurred in flasks with a molybdenum concentration lower than 0.5 γ. With nitrate a great response to molybdenum could be

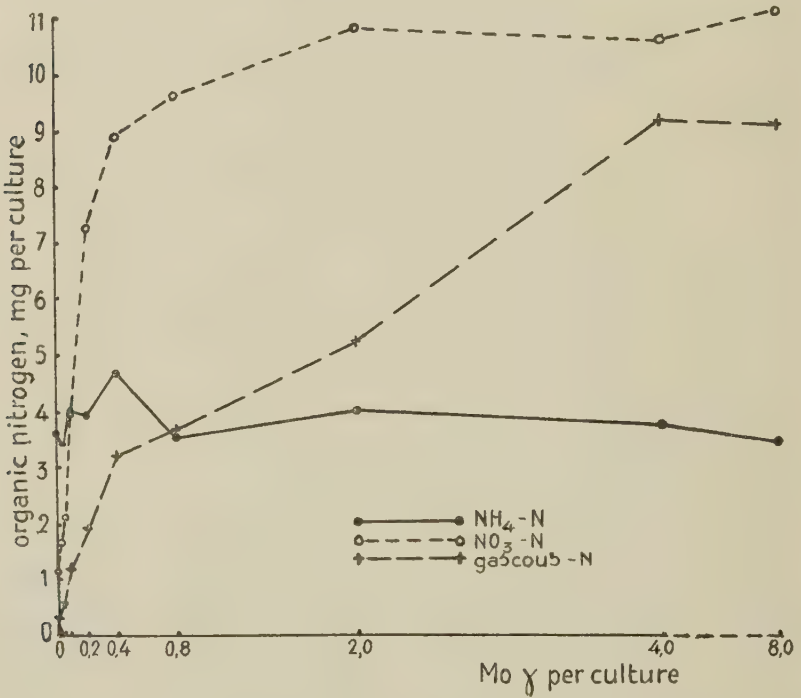

Fig. 4. Effect of molybdenum on the assimilation of different N-compounds by *Azotobacter chroococcum*.

observed, though considerably smaller amounts were needed to reach a normal growth. With ammonium sulphate, however, no influence of the addition of molybdenum could be seen. A somewhat poor growth occurred in all the cultures of this series. Microscopically many *Azotobacter* cells could be seen in the molybdenum-free nutrient solution with ammonium nitrogen; in the nitrate and nitrogen-free solutions, however, only few could be discovered.

Bacterial growth was measured by determining the total organic nitrogen in the cultures. In the set without combined nitrogen the

ordinary Kjeldahl method was used. In the ammonia cultures, however, the ammonia present had to be driven out in vacuo after addition of a boric acid buffer and in the nitrate series the nitrate had to be removed by reducing with $FeCl_2$ and HCl before the organic nitrogen could be determined. The results of this experiment are graphically represented in fig. 4. It is seen that in a medium without combined nitrogen *Azotobacter chroococcum* requires considerably more molybdenum than in a solution with nitrate *). With ammonium sulphate added no response to molybdenum could be observed. This is in good agreement with the results of the above-mentioned experiment with tomato plants according to which the assimilation of nitrate requires traces of molybdenum whereas ammonium compounds can be assimilated in the absence of molybdenum.

The relatively poor growth of *Azotobacter* in the NH_4-solution was possibly caused by an insufficient phosphorus supply. This was concluded from a similar experiment with increasing amounts of phosphate in which solutions with ammonium nitrogen needed about 10 times as much phosphate as those with nitrate and gaseous N_2.

The fact that nitrate assimilation requires less molybdenum than the fixation of N_2 from the atmosphere shows that the molybdenum-requiring reactions involved in nitrate and N_2-assimilation are not identical.

b. Experiments with symbiotic nitrogen-fixing bacteria. In order to know whether or not molybdenum is indispensable for the nitrogen fixation by the nodules of leguminous plants some experiments with peas were carried out.

In preliminary experiments in 1941 and 1942 much evidence was obtained that in the absence of molybdenum the fixation of nitrogen by the nodules of peas was greatly curtailed. Heavy symptoms of nitrogen deficiency were observed. The plants died at an early stage and the yields of seeds and straw were low. Nitrogen determinations in these first experiments, however, were not carried out. In order to confirm these results a third experiment was started in July 1942.

*) That in a medium with nitrate and ammonium nitrogen respectively *Azotobacter* is assimilating these nitrogen compounds and not the N_2 from the air was shown by ammonium and nitrate determinations in the culture solutions.

Pea seeds were germinated in purified quartz sand and on July 7 transplanted into Weck glass jars containing 1.4 l of the following nutrient solution:

H_2O	1 l	$Na_2CO_3.10H_2O$	1.25 mg
KNO_3	0.2 g	$FeCl_3.6H_2O$	25.0 mg
K_2HPO_4	0.125 g	$MnSO_4.4H_2O$	1.0 mg
KH_2PO_4	0.186 g	$ZnSO_4.7H_2O$	0.25 mg
$MgSO_4.7H_2O$	0.250 g	$CuSO_4.5H_2O$	0.25 mg
$CaSO_4.2H_2O$	0.250 g	H_3BO_3	0.25 mg

Of the 16 cultures used, 8 were provided with 100 γ of $Na_2MoO_4.2H_2O$ per jar. All the cultures were inoculated with a suspension of *Rhizobium*. The culture solution was changed three times. No nitrogen was given to the renewed solution, however. All of the other constituents, including molybdenum, were added in concentrations two times as high as those of the medium used initially.

At first no differences between the plants of both series were observed. In both cases many nodules had been formed. In the second part of August, however, the first symptoms of nitrogen deficiency were seen in the absence of molybdenum: a yellow green colour of the leaves and a dying back of the lower ones. These deficiency symptoms progressively worsened (figure 5 and 6). In the presence of molybdenum normal plants were obtained with dark green leaves, indicating an abundant nitrogen supply. The nodules of these plants showed a somewhat pink shade as contrasted with a more pale yellow-brown colour in the absence of molybdenum. The molybdenum-deficient plants died at a much earlier stage than those growing in a complete culture solution. Yields of peas, straw and roots as well as their total nitrogen content are given in table XIV.

TABLE XIV

Effect of molybdenum on the yield and nitrogen fixation of pea plants

$Na_2MoO_4.2H_2O$ per culture, γ	Seeds		Leaves + stems		Roots	
	dry wt. †), g	nitrogen †) mg	dry wt. †), g	nitrogen †) mg	dry wt. †), g	nitrogen †) mg
0	2.35 ± 0.42	77.5 ± 12.4	4.82 ± 0.25	97.5 ± 8.7	1.01 ± 0.04	27.6 ± 1.0
200 *)	5.57 ± 0.34	170.3 ± 12.9	5.92 ± 0.28	114.3 ± 7.4	1.05 ± 0.03	30.2 ± 1.2

*) added after every change of the culture solution.

†) average values of eight separately analysed cultures.

Fig. 5.

Fig. 6.

Fig. 5/6. Effect of molybdenum on pea plants (left without molybdenum, right with 200 γ of Na_2MoO_4 per culture).
The plants in fig. 6 photographed some weeks after those in fig. 5.

From these results it is concluded that pea plants grown with traces of molybdenum have fixed considerably more nitrogen than those with an inadequate molybdenum supply. These nitrogen data, however, may not have been fully representative due to the fact that in the absence of molybdenum the plants died at an earlier date. In order to acquire a more clear picture of the influence of molybdenum upon the nitrogen-fixing-capacity of the nodules, the plants should have been analysed at a time when differences in colour but not in development were observed.

In 1943 a new experiment with pea plants was started on May 27. Six weeks later the first differences in colour between the two series of plants were observed. In both cases nodules had been formed abundantly. Two cultures, grown in the absence of molybdenum, showing a somewhat light green colour of the leaves, and two with a normal molybdenum supply, showing dark green leaves, were harvested and analysed for total nitrogen. The data are given in table XV.

TABLE XV

Effect of molybdenum on the nitrogen fixation by the nodules of pea plants

$Na_2MoO_4.2H_2O$ per culture, γ	Leaves + stems			Roots		
	dry weight, g	nitrogen mg	nitrogen % dry.wt.	dry weight, g	nitrogen mg	nitrogen % dry.wt.
0	4.15	84.9	2.05	1.78	40.4	2.27
0	4.07	79.0	1.94	1.50	32.9	2.19
200	3.22	108.6	3.37	1.35	42.9	3.18
200	4.20	159.9	3.81	1.60	51.9	3.22

These results very clearly show that the fixation of gaseous nitrogen by the nodules of pea plants is reduced when molybdenum has been omitted from the culture solution. Due to this poor nitrogen supply the leaves turn light green and after some time the growth of the plants is stopped.

The view that molybdenum is acting as a catalyst in the fixation of nitrogen in the nodules of legumes is in good agreement with the high molybdenum content found in these tissues. For the molybdenum determinations the *Aspergillus* assay as described on page 101 was employed. Ten or five mg of dried and ground tissue were ashed at 500 600°C and the ash added to molybdenum-free solutions of *Aspergillus*. Table XVI contains the results of this experiment

TAVLE XVI

Molybdenum content of some plant tissues	
Tissue	Molybdenum content p.p.m.
Pea leaves	0.3
Pea roots	2
Pea nodules	4–8
Pasture gras	0.7
Red clover, leaves	2

Molybdenum with reference to agriculture. In large areas in South Australia and New Zealand molybdenum applications are essential for a normal growth of legumes in pastures. Apparently molybdenum stimulates the nitrogen fixation of these plants and this again increases the nitrogen supply of the grasses.

It is highly improbable that an occurrence of molybdenum deficiency will be limited to these Australian soils. It may be expected that in the future in other parts of the world molybdenum-deficient soils will be discovered. As the above-mentioned soils are rather acid and rich in ironstone, a beneficial effect of applying small amounts of molybdenum may be expected in growing leguminous crops in acid soils, which are rich in iron. So far, no response to molybdenum in Dutch soils has been found.

Summary

The effect of molybdenum on the growth of microorganisms and higher plants and on some well-defined biochemical reactions was investigated.

In good agreement with the results of Steinberg it could be shown that *Aspergillus niger* requires small amounts of molybdenum when growing in a culture solution supplied with nitrate nitrogen. With ammonium sulphate as a source of nitrogen the response of the fungus to molybdenum is much smaller. It was shown that this different response of *Aspergillus* to molybdenum was not brought about by a difference in purity of both nitrogen compounds used, nor by a difference in absorption of the molybdenum impurity, but by a considerably higher requirement of molybdenum in a medium with nitrate nitrogen.

The growth-rate curve and the increasing sporulation of *Aspergillus niger* with increasing amounts of molybdenum were used in estimating very small amounts of this element in various materials.

In experiments with 4 strains of denitrifying bacteria the essentiality of molybdenum for the denitrification process was demonstrated. With two strains no growth at all was observed in the absence of molybdenum, with the other two an insignificant denitrification occurred.

An influence of molybdenum on the oxydation-reduction potentials of denitrifying cultures could be seen only when the nitrate in the cultures with molybdenum added was depleted but still present in the o-Mo-cultures.

In culture solution experiments with tomato, barley and oat plants the effect of traces of molybdenum on the growth of these plants was investigated. In good agreement with the results of the experiments with *Aspergillus* and denitrifying bacteria it could be shown that in the green plant as in these microorganisms molybdenum is acting as a catalyst in nitrate reduction.

In experiments with *Azotobacter chroococcum* and leguminous plants the effect of molybdenum on the fixation of gaseous N_2 was studied. When *Azotobacter* was grown in culture solutions without combined nitrogen considerably more molybdenum was required than in media with nitrate nitrogen. With ammonium sulphate as a nitrogen source no response to molybdenum could be observed at all. From these results it was concluded that in *Azotobacter* the molybdenum-requiring reactions involved in nitrate and N_2-assimilation are not similar.

In culture solutions with pea plants the effect of molybdenum on the nitrogen fixation of the nodules was investigated. In the absence of molybdenum as well as in a complete nutrient medium many nodules were formed. In the former case, however, the nitrogen fixation of the nodules was greatly curtailed, resulting in a poor nitrogen supply to the plants. Pronounced nitrogen-deficiency symptoms were shown and the plants died at an early stage. Apparently, in symbiotic as well as in free-living nitrogen-fixing organisms molybdenum is acting as a catalyst in nitrogen fixation.

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